Syndromes, Disorders and Maternal Risk Factors Associated with Neural Tube Defects (IV)

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SUMMARY

Fetuses with neural tube defects (NTDs) may be associated with maternal and fetal risk factors. This article provides a comprehensive review of maternal and fetal risk factors associated with NTDs, such as infertility, periconceptional clomiphene use and assisted reproductive technology, periconceptional folic acid deficiency and effects of folic acid supplementation and fortification on NTD rates, periconceptional vitamin B12 deficiency, single nucleotide polymorphisms and polymorphisms in genes of folate metabolism, and maternal autoantibodies to folate receptors. NTDs associated with maternal and fetal risk factors are an important cause of NTDs. Perinatal identification of NTDs should alert the clinician to the maternal and fetal risk factors associated with NTDs, and prompt a thorough etiologic investigation and genetic counseling. [*Taiwan J Obstet Gynecol* 2008;47(2):141–150]

Key Words: fetal, maternal, neural tube defects, risk factors

Introduction

Neural tube defects (NTDs) have an incidence of 1–2 per 1,000 births and are considered to be a heterogeneous condition resulting from failure of normal neural tube closure between the third and fourth week of embryonic development. The three common types of NTDs are anencephaly, spina bifida, and encephalocele. The uncommon types of NTDs include amniotic band syndrome, limb–body wall complex, cloacal exstrophy or omphalocele-exstrophy-imperforate anus-spinal defects (OEIS) complex and other types of spinal abnormalities. The incidence of NTDs varies with race, geographic variation, socioeconomic classes, nutritional status, and multiple predisposing factors such as single gene disorders, chromosomal abnormalities, teratogens, maternal diabetes, family history of NTDs, and polymorphisms in the genes of folate metabolism. There is considerable evidence that genetics and environmental factors contribute to the etiology of NTDs. Fetuses with NTDs may be associated with maternal and fetal risk factors.

Infertility, Periconceptional Clomiphene Use, and Assisted Reproductive Technology

Wu et al [1] suggested that infertility may be associated with an increased risk of spinal NTDs. In a nested case-control study within the Kaiser Permanente Medical Care Program in Northern California, Wu et al [1] identified 18 cases with spinal NTDs among a birth cohort of 110,624 singleton infants and randomly selected 1,608 cases for controls. They found that case mothers were more likely to have a history of infertility (4/18 *vs.*

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96/1,608; odds ratio, OR, 4.3; 95% confidence interval, CI, 1.01–14.0) and to have had periconceptional clomiphene use (3/18 *vs.* 32/1,608; OR, 11.7; 95% CI, 2.0–44.8). Bánhidy et al [2] found possible interactions among clomiphene treatment, follicular cysts and NTDs. They found an association between the presence of maternal follicular cysts and a higher risk of NTDs in the offspring (7/1,202 *vs.* 88/38,151; crude OR, 3.2; 95% CI, 1.0–10.4), but this association was lost when clomiphene treatment was included among confounders (adjusted OR, 1.7; 95% CI, 0.4–6.9). They also found an association between clomiphene treatment in early pregnancy and a higher risk of NTDs (7/1,202 *vs.* 96/38,151; crude OR, 6.4; 95% CI, 1.3–31.4), but this association was diminished if follicular cysts were included among confounders (adjusted OR, 4.5; 95% CI, 0.7–26.7). The possible association between periconceptional clomiphene use and NTDs is controversial. A positive but mild or indirect association between clomiphene and NTDs has been observed in several reports [1–9]. However, the studies of Mills et al [10], Van Loon et al [11], Greenland and Ackerman [12] and Källén et al [13] have shown that clomiphene may not elevate the risk of NTDs in the offspring of the women using this drug. In a pooled analysis of 10 epidemiological studies, Greenland and Ackerman [12] found that the estimated summary prevalence ratio for the association between NTDs and clomiphene was 1.08, with 95% confidence limits of 0.76 and 1.51. Källén et al [13] studied the outcome after ovarian stimulation without *in vitro* fertilization (IVF) and found that none of the 4,029 infants studied had spina bifida. The possibility that assisted reproductive technology is associated with an increase in major birth defects cannot be excluded based on the current evidence [14–17]. Källén et al [16] studied 16,280 IVF infants born during the period 1982–2001 in Sweden and found that 8% had a congenital malformation, 5% had a relatively severe condition, and an additional risk increase was seen in NTDs, choanal atresia and alimentary tract atresia. In their study, the ORs with 95% CIs for anencephaly, spina bifida and any NTDs were: OR of 7.6, 95% CI of 2.5–7.7; OR of 5.1, 95% CI of 3.4–7.8; and OR of 4.8, 95% CI of 3.3–6.9, respectively.

Periconceptional Folic Acid Deficiency, and Effects of Folic Acid Supplementation and Fortification on NTD Rates

About 70% of NTDs in humans are folate-sensitive or folate-dependent and can be prevented by periconceptional folic acid supplementation [18–21]. In the 1960s, Hibbard [22] and Hibbard and Smithells [23] proposed the association between folic acid metabolism and human embryopathy. In the 1980s, Smithells et al [24] suggested the possibility of NTD prevention by periconceptional vitamin supplementation, and Laurence et al [25], Mulinare et al [26], Bower et al [27] and Mulinsky et al [28] published their results of the prevention of NTD recurrence with periconceptional folate treatment, folic acid-containing multivitamin supplements or dietary folate. In the 1990s, the Medical Research Council (MRC) Vitamin Study Group [18], in a randomized double-blind prevention trial with a factorial design conducted at 33 centers in seven countries, found that folic acid supplementation of 4 mg/day can prevent NTDs. The relative risk (RR) estimate for the women who were at high risk of having a pregnancy with an NTD because of a previous affected pregnancy and were allocated to take folic acid was 0.28, indicating a 72% protective effect (RR, 0.28; 95% CI, 0.12–0.71). Czeizel and Dudás [19] concluded that periconceptional folic acid and vitamin use can decrease the incidence of a first occurrence of NTDs. The results of the MRC Vitamin Study Group [18] and Czeizel and Dudás [19] fueled public efforts to reduce the prevalence of NTDs through educational and food fortification programs. Following the report of the MRC Vitamin Study Group [18], the Centers for Disease Control and Prevention [29] recommended that women who have had an earlier pregnancy affected by an NTD should consume 4,000 μg/day or 4 mg/day of folic acid from the time of trying to become pregnant to the first trimester of pregnancy. In 1992, the US Public Health Service recommended that all women of childbearing age should consume at least 400 μg/day of folic acid [30]. In 1998, the US Food and Drug Administration [31] mandated that folic acid be added to all enriched cereal-grain products at a level of 140 μg/100 g of flour. Werler et al [32] and Shaw et al [33] observed a reduced risk of NTDs among women who had dietary intakes of folate during early pregnancy. Berry et al [34] reported that periconceptional intake of 400 μg of folic acid daily reduced the risk of NTDs in areas of China with high or low NTD rates. The universal protective effect of maternal periconceptional folic acid supplementation is evident by the declining population prevalence of NTDs by 30–50% following folic acid food fortification in many countries [35–45]. The American College of Medical Genetics [46] recommended that:

1. Women capable of becoming pregnant should take 400 μg (0.4 mg) of folic acid daily, in the form of supplement, multivitamin, and/or through fortified foods, in addition to eating a healthy diet. This

is particularly important before conception and through the first trimester of pregnancy.
2. Women who have had a prior NTD-affected pregnancy, have a first-degree relative with an NTD or are themselves affected should be advised to take 4,000 μg (4 mg) of folic acid daily starting at least 1 month and preferable 3 months before conception.
3. The total daily intake of folic acid should not exceed 1,000 μg (1 mg) unless prescribed by a physician, because of a potential concern of masking timely detection of vitamin B12 deficiency.

Bayston et al [47] concluded that there is no evidence of folic acid fortification on the risk of colorectal cancer. Johnston [48] concluded that there is no known evidence that the recommended folic acid supplementation and fortification have caused harm in individuals. However, several reports suggested that all potentially adverse effects caused by a high intake of folic acid from fortified food or dietary supplements should be monitored [49–53]. The possible harms of elevated blood folate concentrations include: (1) influence of DNA and histone methylation; (2) masking of vitamin B12 deficiency; (3) elevated blood concentration of naturally occurring folates and of unmetabolized folic acid; (4) decreased natural killer cell activity; (5) interference with antifolate treatment such as reducing the response to antifolate drugs for malaria, rheumatoid arthritis, psoriasis and cancer; (6) cancer promotion by facilitating progression and growth of preneoplastic cells and subclinical cancers; (7) an increased risk of cognitive impairment and anemia in the elderly with a combination of high folate levels and low vitamin B12 status; and (8) an increased risk of insulin resistance and obesity in the offspring of the pregnant women with a combination of high folate levels and low vitamin B12 status [53].

Periconceptional Vitamin B12 Deficiency

Low maternal serum levels of vitamin B12 have been associated with a higher risk of NTDs [54–62]. Gaber et al [62] and Ray et al [63] found that low vitamin B12 concentration can be associated with an approximately two- to threefold increased risk of NTDs. Ray et al [64] found that about 1 in 20 women may be deficient in vitamin B12 in early pregnancy. Ray et al [63] found that as much as 34% of all NTDs in Canada may be attributable to low maternal vitamin B12 status. Ray et al [64] suggested that adding vitamin B12 to folic acid-fortified foods may help prevent NTDs as well as reduce concern about masking vitamin B12 deficiency and vitamin B12-related neurologic diseases occurring with folic acid food fortification.

Single Nucleotide Polymorphisms (SNPs) and Polymorphisms in Genes of Folate Metabolism

Beaudin and Stover [65] suggested that SNPs in genes of folate-mediated one-carbon metabolism can: (1) influence both maternal and fetal folate status affecting neural tube closure; (2) directly disrupt metabolism resulting in homocysteine accumulation, impaired nucleotide biosynthesis and impaired cellular methylation; and (3) modify genomic and/or cellular responses critical to proper neural tube closure, including cell proliferation, survival, differentiation and migration. Reported SNPs and polymorphisms in genes of folate metabolism associated with NTDs include specific SNPs in genes within folate transport, SNPs in genes within the methionine/homocysteine metabolic cycle, and SNPs and polymorphisms in genes contributing to nucleotide biosynthesis. Several authors have provided a detailed review on the genetic basis of NTDs [65–73].

SNPs in genes within folate transport

Folic acid is absorbed in the proximal small intestine through a carrier-mediated mechanism involving reduced folate carrier (RFC). After entering into the blood stream, folate is transported into the cells through folate receptors (FRs), FR-α, FR-β and FR-γ, and through RFC. There is lack of association between mutations in *FR-α* and *FR-β* and NTDs [74–76]. Rothenberg et al [77] found autoantibodies against FRs in 75% of women with a pregnancy complicated by NTDs but in only 10% of women with a normal pregnancy. The binding of maternal autoantibodies to the FRs on the placental membrane may block the binding of folic acid.

RFC-1 A80G SNP

A polymorphism of A80G in the RFC gene is associated with lower plasma folate status and homocystinemia [78]. The *RFC-1* A80G SNP in the RFC gene has been demonstrated as a genetic risk factor for NTDs, especially under the circumstance of low folate status and mutations in the methylenetetrahydrofolate reductase (*MTHFR*) gene [79–82].

SNPs in genes within the methionine/homocysteine metabolic cycle

MTHFR C677T SNP

An *MTHFR* C677T SNP is associated with a reduction of MTHFR enzymatic activity and elevated levels of

plasma homocysteine. The *MTHFR* C677T SNP in the *MTHFR* gene has been demonstrated as a genetic risk factor for NTDs [68,83–93]. In a meta-analysis, Blom et al [68] concluded that there is a moderately elevated risk for NTDs in maternal and fetal *MTHFR* 677TT homozygous genotypes (60% increase; OR, 1.6; and 90% increase; OR, 1.9, respectively), and a mildly elevated risk for NTDs in maternal and fetal *MTHFR* 677CT heterozygous genotypes (10% increase; OR, 1.1; and 30% increase; OR, 1.3, respectively).

Methylation hypothesis

The *MTHFR* 677TT genotype produces the lowest MTHFR activity, higher concentrations of 5,10-methylenetetrahydrofolate (5,10-methylene THF) and 10-formyltetrahydrofolate (10-formyl THF), increased DNA synthesis, lower concentrations of 5-methyltetrahydrofolate (5-methyl THF), and consequently decreased methylation which may be more pronounced under the condition of low folate status [68]. The *MTHFR* 677CC genotype produces the highest MTHFR activity, lower concentrations of 5,10-methylene THF and 10-formyl THF, decreased DNA synthesis, higher concentrations of 5-methyl THF, and consequently increased methylation [68]. The *MTHFR* 677CT genotype produces an intermediate effect on DNA synthesis and methylation [68]. Blom et al [68] suggested the methylation hypothesis in that the *MTHFR* C677T SNP results in an increased NTD risk through disruption of the methylation of lipids, DNA and protein during early embryogenesis, and that folate prevents NTDs by increasing methylation of various molecules that are essential to cellular processes.

MTHFR A1298C SNP

An *MTHFR* A1298C SNP is associated with a reduction of MTHFR enzymatic activity but has no effect on homocysteine plasma levels [94–97]. Combined SNPs of *MTHFR* C677T and *MTHFR* A1298C have been demonstrated to be associated with an increased risk for NTDs [89,94,98,99]. De Marco et al [100] reported that *MTHFR* A1298C SNP was a genetic determinant of NTD risk in Italy. To date, only the study reported by De Marco et al [100] found an association between *MTHFR* A1298C SNP and NTDs. The evidence regarding the effect of *MTHFR* A1298C SNP on NTDs is limited. van der Linden et al [71] concluded that *MTHFR* A1298C SNP is unlikely to be a risk factor for NTDs.

MTR A2756G SNP

Methionine synthase (MTR) converts folate and homocysteine to tetrahydrofolate and methionine. The association between *MTR* A2756G SNP and NTDs is inconclusive. Concerning *MTR* A2756G SNP in relation to NTDs, some studies found an increased NTD risk [101–104], other studies found no association [100, 105–108], and one study found a decreased NTD risk [87]. van der Linden et al [71] concluded that if there is a relationship between the *MTR* A2756G SNP and an NTD risk, it is at most a rather moderate association.

MTRR A66G SNP

The enzyme methionine synthase reductase (MTRR) activates MTR. The role of MTRR variants in NTDs has yet to be established. Concerning the *MTRR* A66G SNP in relation to NTDs, some studies found an increased NTD risk [57,71,103,109], whereas other studies found no association [104,108,110]. In a meta-analysis of eight relevant studies on the *MTRR* A66G SNP and maternal NTD risk, van der Linden et al [71] found that the *MTRR* A66G SNP genotype in mothers was associated with an overall 48% increase in NTD risk (OR, 1.48; 95% CI, 1.00–2.19) and concluded that the *MTRR* A66G SNP genotype seems to be an NTD risk factor.

BHMT rs3733890 SNP

Betaine-homocysteine methyltransferase (BHMT) remethylates homocysteine to methionine with a betaine cofactor. Boyles et al [111] found that the *BHMT* rs3733890 SNP was significantly associated with NTDs, particularly when mothers were receiving preconceptional folate or parents preferentially transmitted the *MTHFR* rs1801133 T allele. Boyles et al [111] hypothesized that: (1) the *BHMT* polymorphism could create a highly efficient variant that causes the metabolic cycles to overfunction when combined with high folate levels; (2) a gene–gene interaction between *BHMT* and *MTHFR* could require polymorphisms in both genes for NTDs; or (3) additional correlated factors are involved and undetectable in their study samples.

SNPs and polymorphisms in genes contributing to nucleotide biosynthesis

MTHFD1 G1958A SNP

The trifunctional enzyme, C1 synthetase or MTHFD1 (methylenetetrahydrofolate dehydrogenase [MTHFD]/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase), is a trifunctional nicotinamide adenine dinucleotide phosphate-dependent cytoplasmic enzyme that has three enzymatic properties: (1) 10-formyl THF synthetase that reversibly converts THF to 10-formyl THF, (2) methyl THF cyclohydrolase that reversibly converts 10-formyl THF to 5,10-methenyl THF, and (3) MTHFD that reversibly converts 5,10-methenyl THF to 5,10-methylene THF [112]. 10-formyl THF and 5,10-methylene THF are the donor cofactors

for *de novo* purine and pyrimidine biosynthesis, respectively. *MTHFD1* G1958A SNP results in the substitution of an arginine (R) by a glutamine (Q), or R653Q, within the 10-formyl THF synthetase domain of the MTHFD1 enzyme. Hol et al [113] found that the *MTHFD1* G1958A SNP had a similar frequency among the NTD patients and normal individuals and did not influence the plasma homocysteine level. Brody et al [114] found an association of QQ homozygosity in *MTHFD1* G1958A (R653Q) SNP with mothers of children with NTD in a study of Irish population. They also found that the plasma folate, red blood cell folate and homocysteine levels in 653QQ mothers were not altered. Brody et al [114] predicted that mothers with the QQ homozygosity in *MTHFD1* G1958A (R653Q) SNP have a 1.5- to 2-fold risk of having an NTD-affected pregnancy compared with the controls. Brody et al [114] found QQ homozygosity to be overrepresented in mothers of children with NTD and underrepresented in children with NTD; they concluded that genetic variation in the *MTHFD1* gene is associated with an increase in the genetically determined risk that a woman will bear a child with NTD and that the gene may be associated with decreased embryo survival. Parle-McDermott et al [115] confirmed that the R653Q polymorphism of trifunctional MTHFD1 enzyme is associated with maternal risk for NTDs in the Irish population. De Marco et al [116] identified *MTHFD1* 1958GA and *MTHFD1* 1958AA genotypes in NTD patients from an Italian population and suggested that *MTHFD1* 1958GA SNP is a risk factor for NTDs. In the absence of effects on folate and homocysteine levels, the *MTHFD1* G1958A SNP may affect the neural tube closure via alterations in a folate-dependent anabolic pathway other than the methylation mechanism, or via alterations in the nucleotide pools available for DNA synthesis [65].

cSHMT C1420T SNP

Serine hydroxymethyltransferase (SHMT) is associated with thymidylate biosynthesis. SHMT catalyzes the reversible conversion of serine and THF to glycine and 5,10-methylene THF. Heil et al [117] found that the cytosolic isoform of the *SHMT* gene polymorphism *cSHMT* C1420T SNP was not associated with an NTD risk in mothers of patients and that the mothers with *cSHMT* 1420CC genotype had significantly elevated plasma homocysteine levels and decreased erythrocyte and plasma folate levels. Relton et al [99] demonstrated a protective effect associated with the T allele in mothers of patients. van der Linden et al [71] concluded that the *cSHMT* C1420T SNP is at most a minor risk factor for NTD risk. However, the efficiency of nuclear folate metabolism is likely to be modified by the *cSHMT* C1420T polymorphism. Woeller et al [118] found that *cSHMT* C1420T SNP impaired the UBC9-cSHMT interaction and inhibited cSHMT small ubiquitin-like modifier (SUMO)-ylation *in vitro* resulting in cSHMT accumulation in the cytoplasm and impairment of homocysteine remethylation pathway and folate-dependent *de novo* thymidylate biosynthesis pathway in the nucleus. A synergistic gene–gene interaction between *cSHMT* C1420T SNP and *MTHFR* C677T SNP has been observed in an epidemiologic study of cardiovascular disease risk [119]. Lim et al [119] found the increased risk of cardiovascular disease associated with *MTHFR* 677CT and *MTHFR* 677TT genotypes was of greater magnitude among men with the *cSHMT* 1420TT genotype.

TS 28-bp thymidylate synthase enhancer region (TSER) and TS 6-bp deletion in the 3′ untranslated region (UTR)

Thymidylate synthase (TS) catalyzes the conversion of deoxyuridylate to thymidylate and is associated with thymidylate biosynthesis. *TS* contains a 28-bp tandem repeat in the *TS* promoter enhancer region (TSER) in the 5′ UTR. The tandem repeat polymorphism affects the expression of the enzyme. The three repeats (3R) in the 5′ UTR confers higher translation efficiency than the two repeats (2R) in the 5′ UTR *in vitro* [120]. *TS* mRNA with a 3R sequence has higher translation efficiency than that with the 2R sequence *in vivo* [121,122]. Trinh et al [123] observed that the TSER 3R3R genotype was associated with reduced plasma folate and elevated plasma homocysteine levels among the Chinese population of Singapore with low dietary folate intake. Wilding et al [124] found that *TS* repeat polymorphism in TSER was not associated with an NTD risk in the northern United Kingdom population. However, Brown et al [125] observed that the *TS* tandem repeat polymorphism was not associated with homocysteine concentrations in the northwestern European population and that the TSER 3R3R genotype was not a determinant of homocysteine. The 6-bp deletion in the 3′ UTR of *TS* affects RNA stability and translation [126]. Volcik et al [127] found that the NTD risk increased by fourfold with the TSER 2R2R genotype, by threefold with the undeleted homozygous genotype of 3′ UTR++ and by more than fourfold with the combined TSER 2R2R and 3′ UTR++ genotypes in non-Hispanic whites but not in Hispanic whites, African-Americans or Asian-Americans.

DHFR intron-1 19-bp deletion

Dihydrofolate reductase (DHFR) plays a role in regenerating THF from dihydrofolate during folate-linked synthesis of thymidine. Johnson et al [128] suggested that maternal homozygosity for the 19-bp deletion

allele polymorphism in intron 1 of the *DHFR* gene is a risk factor for spina bifida. However, van der Linden et al [129] did not find an association between *DHFR* intron-1 19-bp deletion and NTDs. On the contrary, Parle-McDermott et al [130] demonstrated that the *DHFR* intron-1 19-bp deletion may be a protective NTD genetic factor by increasing *DHFR* mRNA levels in pregnant women. Stanisławska-Sachadyn et al [131] confirmed the findings of Parle-McDermott et al [130] and suggested that the *DHFR* deletion/deletion homozygotes have increased serum and red blood cell folate concentrations and may therefore be at decreased risk of having NTD offspring.

Maternal Autoantibodies to Folate Receptors

Folic acid is absorbed in the proximal small intestine through a carrier-mediated mechanism involving RFC. After entering into the blood stream, folate is transported into the cells through folate receptors (FRs), FR-α, FR-β and FR-γ, and through RFC. Rothenberg et al [77] found autoantibodies against FRs in 75% of women with a pregnancy complicated by NTDs but in only 10% of women with a normal pregnancy. The binding of maternal autoantibodies to the FRs on the placental membrane may block the binding of folic acid. The occurrence of the autoantibodies may explain the beneficial effect of periconceptional folate supplementation. The 75% of the index subjects is similar to the 70% decrease in NTDs by periconceptional folic acid supplementation. Folic acid has a high affinity for the FRs. Folic acid can displace an autoantibody with a low affinity for the FRs. This study suggests that autoantibody-mediated blocking of cellular folate uptake by the FRs can be bypassed by folic acid.

Conclusion

This article provides a comprehensive review of maternal and fetal risk factors associated with NTDs, such as infertility, periconceptional clomiphene use and assisted reproductive technology, periconceptional folic acid deficiency, and effects of folic acid supplementation and fortification on rates of NTDs, periconceptional vitamin B12 deficiency, SNPs and polymorphisms in genes of folate metabolism, and maternal autoantibodies to folate receptors. NTDs associated with maternal and fetal risk factors are an important cause of NTD. Perinatal identification of NTDs should alert the clinician to the maternal and fetal risk factors associated with NTDs, and prompt a thorough etiologic investigation and genetic counseling.

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