Folate-responsive birth defects: of mice and women1,2

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The recognition that periconceptional folate supplementation in addition to normal dietary folate intake reduced the incidence of neural tube defects (NTDs), one of the most common birth defects, has led to mandatory folate fortification in the United States and ~50 other countries (1, 2). Fortification programs have reduced the incidence of NTDs by ~20–80%, with the largest reductions occurring in populations with the highest pre-fortification rates (3). The benefits of folate fortification in reducing birth defects and in reducing the incidence of folate deficiency in the general population have been clearly shown (3, 4). However, concerns have been raised about possible adverse effects of high folate intake on segments of the population other than women of childbearing age. The level of fortification in the United States was estimated to provide, on average, 100 µg additional folic acid daily with only a very small proportion of the population receiving >1 mg to prevent masking of symptoms of vitamin B-12 deficiency (anemia), primarily in the elderly population. More recently, concerns have been raised that high folate may promote tumor growth (5). Although poor folate status is a risk factor for various cancers, some studies have suggested that high folate intakes have increased the incidence of some cancers, and it has been speculated that high folate may increase the growth rate of existing neoplasms.

It would be beneficial if it were possible to identify those women most at risk of NTDs. However, the reason why folate influences NTD rates is not known. NTDs are a low-penetration condition influenced by environmental factors such as folate intake and genetic factors such as polymorphisms in folate genes, among others. A large number of human gene variants have now been screened for NTD risk. The most well-established risk factor is a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR 677C>T) gene (6). However, the vast majority of fetuses that are homozygous for the 677T allele do not develop NTDs. Mouse models for NTDs have the potential for providing more mechanistic information. A large number of mouse models for birth defects have been described, but few are folate responsive. Disruption of the mouse Folbp1 gene, which encodes a folate receptor involved in folate transport, results in spina bifida and embryonic lethality; both can be prevented by very high folate, indicating, in this model at least, that an impairment of folate-dependent one-carbon metabolism is sufficient to generate the NTD phenotype (7).

In this issue (8) and in a separate recent article (9) of the Journal, Beaudin et al report on the first folate-responsive mouse model for NTDs due to a disruption in a gene encoding an enzyme directly involved in one-carbon metabolism. Mice lacking Shmt1, which encodes cytosolic serine hydroxymethyltransferase (cSHMT), developed exencephaly, a form of anencephaly, when fed a diet deficient in folate and choline but did not develop spina bifida (9). Choline deficiency was used because of epidemiologic evidence suggesting an effect on NTDs (10). Follow-up studies showed that this phenotype was due to reduced folate and not to reduced choline (8). Folate-dependent one-carbon metabolism is required for the synthesis of precursors for DNA synthesis (thymidylate and purines) and for methionine synthesis (homocysteine remethylation) and consequently for methylation reactions involved in a host of processes including epigenetic events (11). Because choline is also involved in methyl group status and homocysteine remethylation, their data suggest that the NTD phenotype and folate responsiveness of this mouse model were due to a defect in DNA replication rather than a defect in methylation reactions. cSHMT in the cytosol can provide one carbons for methionine synthesis. However, during the S phase of the cell cycle, it translocates to the nucleus together with the 2 other folate-dependent enzymes in the thymidylate synthesis cycle, thymidylate synthase and dihydrofolate reductase, to support DNA replication (12).

The involvement of defective DNA synthesis in NTD generation was further reinforced by the authors’ studies in mice with the Splotch mutation in the Pax3 gene (9). Pax3 encodes a transcription factor and Splotch has high penetrance for NTDs, mainly spina bifida but also some exencephaly. The Splotch mutant had previously been shown to be impaired in de novo thymidylate synthesis and that its NTD phenotype was folate responsive and could also be rescued with thymidine (13, 14). In the current studies, by using embryonic fibroblasts from the Pax3 mutants, the impairment in de novo thymidylate synthesis was reconfirmed and, interestingly, de novo purine synthesis was also impaired. Methylation potential, as measured by the S-adenosylmethionine:S-adenosylhomocysteine ratio, was unimpaired and actually increased. Deletion of Shmt1 on the Pax3SpSp background exacerbated the NTD phenotype, and the effect was enhanced by combined maternal folate and choline deficiency. Embryonic fibroblasts from Splotch mutants expressed higher

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amounts of cSHMT protein and lower amounts of thymidylate synthase, whereas Pax3 amounts increased in embryos from Shmt1-mutant mice fed the folate- and choline-deficient diet. These data indicate that, in these mouse models at least, the NTD phenotype shares a common etiology of defective DNA synthesis and the genetic disruptions appear to be cross-regulating the same subset of enzymes.

How relevant is this to human NTDs? No genetic variants in the human folate receptor α gene (equivalent to mouse Folbp1), in the cSHMT gene or in the Pax3 gene have been identified that are risk factors for human NTDs. Conversely, mice with deletions in the MTHFR gene do not develop NTDs. Although some may question the usefulness of mouse models for identifying risk factors for human NTDs, there has been a paucity of studies investigating posttranscription risk factors, which may also play a role in NTD etiology. For example, some studies have found that maternal circulating antibodies to the folate receptor are a risk factor for NTDs (15), although other studies have been unable to confirm this (16). cSHMT is also an example of an enzyme that is regulated posttranscriptionally by a variety of factors (17).

It is reasonable to suppose that the underlying mechanism of defective DNA replication suggested by the mouse models of NTDs would be shared by human NTDs. This mechanism is particularly attractive because closure of the neural tube would require coordinated regulation of cell division of layers of cells growing at different rates. Whereas a multitude of factors can cause defective DNA synthesis, the folate responsiveness of most human NTDs would limit the possibilities to a much smaller subset.

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REFERENCES