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Gene-Environment Interactions, Folate Metabolism and the Embryonic Nervous System

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Abstract

Formation of brain and spinal cord requires the successful closure of neural ectoderm into an embryonic neural tube. Defects in this process result in anencephaly or spina bifida, which together constitute a leading cause of mortality and morbidity in children, affecting all ethnic and socioeconomic groups. The subject of intensive research for decades, neural tube defects (NTDs) are understood to arise from complex interactions of genes and environmental conditions, though systems-level details are still elusive. Despite the variety of underlying causes, a single intervention, folic acid supplementation given in the first gestational month can measurably reduce the occurrence of NTDs in a population. Evidence for and the scope of gene-environment interactions in the genesis of NTDs are discussed. A systems-based approach is now possible toward studies of genetic and environmental influences underlying NTDs that will enable the assessment of individual risk and personalized optimization of prevention.

Keywords

Neural tube defects; Genetics; Epigenetics; Folic acid; Gene networks; Pathway interactions; Spina bifida; Anencephaly; Mouse models

The nervous system first emerges as a specialized sheet of ectodermal cells called the neural plate. During the first 30 days of human development, or 10 days in the mouse, the edges of this plate--the neural folds--rise from the horizontal plane as cells in the plate and underlying mesoderm divide, change shape and move. When the neural folds bend and join in the midline, they form the neural tube that gives rise to the brain and spinal cord. Neural tube defects (NTDs), or failure of this neurulation process, are among the most common serious birth defects, occurring in 0.5 to 10 per thousand live births, depending on the population studied (1). Despite extensive prevention efforts using folic acid supplementation, NTDs remain second only to congenital heart anomalies in their prevalence worldwide. Failure of cranial neurulation produces first exencephaly, which is commonly seen in mice because of their 21 day gestation, while the nine-months gestation of human fetuses results in anencephaly as exposed brain tissues are broken down. After neurulation, skull defects may lead to encephalocele or extrusion of meninges through the opening with or without brain tissue. NTDs at spinal levels include spina bifida, which may manifest as rachischisis, or completely open neural tube that may extend part way or throughout the rostral-caudal levels of the neuraxis. Spinal NTDs may also appear as meningocele, in which the meninges extrude through the defect, or myelomeningocele, in which nerve roots and/or spinal cord extend into

the defect of the dorsal spinal column. Despite these different features, either or both spina bifida and anencephaly may occur in the same family or among mouse siblings, reflecting the stochastic nature of where and when neurulation may fail in an individual.

Over 20 years of clinical investigation and experimental study of mouse NTD models indicate that this disorder arises from a combination of factors including complex genetic and gene-environment interactions (2–4). Indeed, over 150 genes have been identified in the mouse that when mutated can cause NTDs (5). In view of the complexity of genetic interactions that can lead to neurulation failure, it is surprising that periconceptional supplementation with a simple vitamin, folic acid, can reduce NTD occurrence by 70% or more (6–9). It may well be that folic acid has the ability to impact such a broad swath of NTDs because the folate metabolic pathway contributes to so many physiological processes ranging from nucleotide biosynthesis, needed for cell proliferation, to generation of pterin cofactors impacting biochemical reactions, and generation of the principal methyl donor, s-adenosyl methionine (SAM), that participates in methylation of DNA and histones modulating gene expression, methylation of proteins and lipids (Figure 1) (10–12). Here, we will discuss some of the ways in which developmental gene networks may intersect with folate metabolism and how this may bear on the expression of NTDs.

Complex Genetics Leading to Failed Neural Tube Closure

It has been appreciated for over 30 years that NTDs in humans are not monogenic malformations but rather are caused by the interplay of multiple genes as well as gene-environment interactions (13). NTD concordance rates among same-sex twins (presumed monozygotic) are significantly increased (to 6.8%) supporting a genetic contribution (14). Moreover, compared to a general population incidence of 1/1000, the recurrence NTD risk in a family with one affected child increases to 1.8/100, but does not approach the 1/4 recurrence risk of an autosomal recessive mutation with complete penetrance. Studies have documented the elevated occurrence rate of NTDs in females compared to males (3:1) and significantly higher incidence of consanguinity among parents of infants with NTDs (15,16). These and numerous other studies indicate prominent genetic and heritable contributions leading to failure of neurulation that may well involve the concerted action of multiple genes to manifest the disorder.

The complexity of the genetic underpinnings of neurulation is clearly indicated by NTD-prone mouse lines in which modifier loci have been detected and in some instances identified. One classic example is the *Curly tail (ct)* mouse, in which spina bifida is associated with a primary risk gene and at least three distinct modifier loci (17). The hypomorphic mutation primarily responsible for NTDs in *ct/ct* embryos occurs in a transcription factor, *Grhl3* (18). Another mutant with demonstrated genetic interactions is the *SELH/Bc* mouse, for which four loci have been mapped that collude to result in exencephaly (5,19). Moreover, there are examples in the literature of digenic mutations—i.e., mutations of two genes—that collaborate to produce NTDs in the mouse (5). These digenic interactions affect processes ranging from cell polarity (*Cobl/Vangl2*, *Dvl1/Dvl2*, *Fzd3/Fzd6*, *Vangl2/Celsr1*, *Vangl2/Scrb*, *Vangl2/Ptk7*), actin cytoskeletal regulation (*Enah/Vasp*, *Enah/Pfn1*), cell interaction-adhesion (*Gja1/Gja5*, *Itga1/Itga6*), intracellular protein transport (*Snx1/Snx2*), to intracellular signaling (*Jnk1/Jnk2*, *Prkaca/Prkacb*) or transcription regulation (*Msx1/Msx2*, *Rara/Rarg*). Thus genetic interactions that confer increased NTD risk do occur in mice and can be anticipated in humans as well.

The sheer numbers of mutant genes in the mouse that predispose to NTD are another indicator that multiple gene interactions underlie human spina bifida and anencephaly. Over 200 NTD-prone mouse mutants have been identified, of which more than 150 responsible mutant genes are known (3,5). The functional categories of NTD genes speak to the pathways that contribute

to neurulation. These include transcription factors such as *PAX3*, *Cart1*, *Cited2*, *Grhl3*, genes in signaling pathways like Wnt (*Wnt3a*, *Lrp6*, *Fzd6*, *Dvl1/2*) and Shh (*Gli3*, *ptch*, *Fkbp8*), chromatin remodeling and DNA methylation pathway genes (*Hdac4*, *Sirt1*, *Gcn5*, *Dnmt3b*), genes involved in folate transport (*Folr1*, *RFC1*), cell cycle regulation (*Phactr4*) (20), apoptosis--either a lack of programmed cell death (*Jnk1/Jnk2*, *Apaf1*, *Casp3*, *Casp9*, and *Cycs*) or excessive apoptosis (*Bcl10*, *Map3k4*), and cytoskeletal regulation (*Shroom3*, *Marcks*, *p190RhoGAP*).

The number and variety of these NTD-associated genes in the mouse are reaching a threshold needed to cluster them and construct pathway relationships that may prove mechanistically if not diagnostically useful. For example, NTD genes *Cobl*, *Vangl*, *Celsr*, *Scrb*, *Ptk7*, *Fuz*, *Fzd*, *Dvl* etc., can be connected through the planar cell polarity pathway (PCP) of non-canonical Wnt signaling. They encompass receptors and downstream effector molecules in a particular developmental signaling system that modulates cell shape and dynamic characteristics of the neuroepithelium. A common feature of animal models bearing mutations in these genes is their demonstrated deficit in convergent extension of the neuraxis (21–27). Abnormal convergent extension is also apparent in human fetuses with craniorachischisis in which the cervical-thoracic or cervical-lumbar extent or the neuraxis is foreshortened. These phenotypes will probably involve abnormalities in PCP related genes. However, craniorachischisis comprises a rather small proportion of NTD cases.

Mutations of PCP genes may also produce milder human phenotypes than rachischisis. A search for rare variant alleles of human *VANGL1* genes led to the identification of three rare SNPs in *VANGL1* in patients with lumbar spina bifida (28). *In vitro* analysis showed that at least one of these SNPs abolished the ability of *VANGL1* to bind to *DVL1* (28). The presence of an inherited variant *VANGL1* allele in an unaffected family member suggests that other factors must collaborate with this mutation to result in an NTD.

Another example of emerging molecular network interactions is found in the cross-talk between Shh and Wnt pathway-related genes in the production of NTDs. Shh signaling has been associated with NTD through mutations in *ptc* (29), *Gli3* (30) and the intraflagellar transport (ITF) proteins that make up primary cilia including *ITF172* and *ITF88* (20). It is important to note that virtually all cells in mammals, not just specialized epithelia, have a primary cilium. Mouse ITF proteins identified in NTD mouse mutants have effects on both activator and repressor functions of Gli as well as regulate patch and Gli protein levels (31). Involvement of the primary cilium in Shh signaling suggests that this structure may serve to integrate Shh and Wnt pathways, since primary cilia endow cells with polarity, and disruption of the ciliary gene *Bbs4* interferes with *Vangl2* function and PCP (32), possibly through *Vangl* effects on *Fzd* receptor function (25) and/or *Vangl* interaction with *Dvl* (28).

Recent evidence implicates another transcription factor, *Msx1*, as a possible molecular node of intersection between Shh and Wnt pathways. *Msx1* has been shown to induce *Wnt1* expression in the dorsal neural tube and *Msx1/Msx2* loss of function--associated with NTDs--entirely abolishes *Wnt1* expression in the dorsal diencephalon (33) but does not affect *Wnt1* expression in the neural crest (34). During limb development, *Msx1/Msx2* have been identified as targets and downstream effectors of the truncated Gli3R repressor (35). In *Shh* null mutants, *Msx* genes are overexpressed, increasing apoptosis in the limb. It appears then that, depending on the level and location within or around the neural tube, *Msx* genes may be regulated by Shh signaling and in turn impact Wnt expression.

The large and growing collection of implicated genes and the numerous ways in which genetic interaction may occur among them pose a particular challenge for efforts to assess NTD risk in a clinical population. This task becomes even more complex--though in some ways more

understandable--when the impact of epigenetic influences is also considered. This is evident in the relationship between various metabolic pathways, notably folate metabolism, and NTDs.

Epigenetic Influences on Neurulation Outcome

Environmental influences contributing to NTD have long been recognized (14,36). These include maternal factors like diabetes in which both hyperglycemia and associated hyperinsulinemia are risk factors for NTDs (37). Maternal obesity and pre-pregnancy weight gain are also associated with increased NTD risk (38,39). In addition, maternal periconceptional increases in simple sugars can elevate the glycemic index, associated with increased NTD risk even in non-diabetic women (40). In animal models, exposure of rat embryos to a hyperglycemic environment induces dysmorphogenesis associated with biochemical markers of oxidative stress and inositol depletion (41).

Certain drugs taken in the periconceptional period are also associated with increased NTD risk. Medicinal retinoids including isotretinoin (Accutane) are derivatives of retinoic acid (vitamin A), a potent morphogen that can either induce or prevent NTDs in animal models, depending on the genetic setting and nutritional status (42,43). Both hypovitaminosis A and retinoid medications have been linked to human birth defects including NTDs (44). Retinoic acid can reduce the NTD occurrence in mouse mutants like *Spotch* (*Pax3*) and *Curly tail* (*Grhl3*), implying a significant gene-nutrient interaction (45,46). Moreover, retinoic acid can impact expression of *Vangl1* and *Vangl2* in mouse embryos, implicating an influence of retinoids on PCP pathways (47).

Most if not all anti-epileptic drugs (AEDs) are known to have teratogenic effects (48). Carbamazepine (CBZ, Tegretol) has been associated with NTDs in 0.5 to 1% of pregnancies in women taking the medication, in addition to increases in cardiovascular anomalies and cleft lip/palate. Among AEDs, valproic acid (VPA, Depakote) has one of the strongest associations with NTDs, raising the risk of spina bifida or anencephaly more than ten fold to 1–2% of pregnancies conceived while on the drug (49). A number of mechanisms have been postulated, but there is a general consensus that genetic predisposition to its teratogenic effect is required for VPA to promote NTDs (36,50). Among potentially teratogenic mechanisms associated with VPA are increased homologous recombination and generation of reactive oxygen species (51), VPA-induced changes in gene expression (52–54), and epigenetic reprogramming through direct inhibition by VPA of histone deacetylases (HDACi) (55,56).

Perhaps the most compelling case for gene-environment interactions is the association of NTDs with vitamin deficiency (57) and the capacity for maternal folic acid supplementation to reduce the occurrence rate of anencephaly or spina bifida in some populations (6,58,59). Yet, after more than two decades of research, the mechanism(s) by which folic acid can intervene to prevent NTDs are incompletely understood (3,4,60). A significant challenge has been the illumination of which gene mutation(s) confer NTD risk and how/what part of the folic acid metabolic pathway can compensate for a given deficit. At present, three main types of single gene variant effects have been described, including those that alter folate status (intracellular folate concentrations), that impact folate utilization and/or metabolism, and that affect both folate status and metabolism or utilization (11).

Consistent with clinical observation, experimental animal data indicate that intracellular folate deficiency predisposes to NTD. For example mice lacking the folate binding receptors *Folr1* or *RFC1* needed for folate transport into cells display severe embryopathy and NTDs that can be largely ameliorated with folic acid in high doses (61,62). There have also been reports of anti-folate receptor antibodies capable of blocking folate uptake detected in 9 of 12 mothers (75%) bearing NTD-affected fetuses compared to 10% of controls (63). An attempt to replicate this study in a much larger Irish cohort found folate receptor antibodies equally among women

with normal and NTD-affected pregnancies (64). However, that study included some samples taken as long as 10 years after the pregnancy in question, weakening interpretation. When measured in serum samples taken from women in mid-gestation, anti-folate receptor antibody titers were significantly higher at 15–18 weeks among pregnancies resulting in neurulation failure (65). If an autoimmune mechanism is operant in humans, it may require a specific set of circumstances to negatively impact neurulation. With the exception of a common variant SNP in the RFC1 gene (66), there have not been any coding sequence variants in folate receptor genes associated with human NTDs and depletion of folate in experimental animals has not resulted in NTDs (4,67,68). Nevertheless, the dramatic embryopathy associated with aggressive folate depletion in animal models and with the folate receptor knockout mice suggests that in humans, variant genes that severely impair folate transport or intracellular metabolism may be incompatible with life and would induce early embryonic lethality. Moreover, knockout of *Mthfr* alone is insufficient to produce NTD in mice (69). Thus, errors of folate transport into cells or of folate metabolism may not cause NTDs without some additional predisposing mutation.

Although the earliest associations between NTDs and folic acid were made in pregnant women with folate deficiencies, folic acid supplementation probably most often reduces NTD occurrence in the absence of overt folate deficit (70,71). Instead, individual partial blocks or inefficiencies in the folic acid metabolic pathway are most likely to promote NTDs, though such changes are probably insufficient in isolation to precipitate neurulation failure. The requirement for some additional interaction is suggested by the existence of reports of either absent or positive NTD-associations with maternal and/or fetal folate metabolism gene variants. One such example is the thermolabile, 677C>T allele of *MTHFR*, which reduces the reductase activity over 50% in homozygous 677T/T individuals. This allele is generally considered the strongest candidate for underlying human folate-responsive NTDs, reflected in the positive association of both maternal and fetal alleles with NTDs (10,72). However, there are also examples reporting lack of association between the 677T/T status and NTD in humans, even among populations in which the allele is prevalent (73). The 677T allele in itself did not raise the risk of NTD in one case control study, but it strengthened the NTD risk conferred by a BHMT allele (74), supporting the case for both gene-gene interactions and the importance of the remethylation/methylation aspect of the folate pathway in neurulation (75).

Critical physiologic processes in which the folate metabolic pathway participates include biosynthesis of purines and thymidylate needed for DNA replication and transcription into RNA, production of bipterin co-factors used in important biosynthetic pathways (12,76–79), and the methionine cycle generating S-adenosyl methionine (Ado-met), the most ubiquitous methyl donor in cells for methylation of DNA, histones/chromatin, proteins and lipids (11, 80). NTD risk associated with folate metabolism-related polymorphisms may depend on the presence of other NTD-risk alleles involving genes not immediately recognized as part of the folate pathway, as evidenced among the few NTD-prone mouse models that have been tested and found to respond to folic acid supplementation (3,4). For example, protection against NTD in *Pax3* mutant mice was conferred by either folic acid or thymidine administration to *Sploch* embryos *in vitro* or *in utero* (81,82). *In vitro*, deoxyUridine suppression experiments in the *Pax3* mutant embryos identified a block in the thymidylate synthesis portion of the folate pathway (81). Moreover, folic acid deficiency increased the occurrence of NTD in *Pax3* mutant mice (83). While folate depleted mutant embryos did show a reduced Ado-met/Ado-hcy ratio, indicating a reduced methylation capacity of the folate pathway, the null embryos did not however display a reduction in global DNA methylation. This led the authors to postulate the most important influence of folic acid on *Pax3* mutants was the promotion of nucleotide biosynthesis and support of embryonic growth.

The first evidence for interaction between a crucial developmental signaling pathway and folate metabolism was the ability of moderate dietary folic acid supplementation to reduce the occurrence of exencephaly in the *Crooked tail* (*Cd*) mouse, which bears a point mutation in *Lrp6* (84,85). *Lrp6* and its paralog *Lrp5* encode co-receptors for Fzd1-9 and are required for canonical Wnt signaling. The LRP6^{Cd} protein in mouse embryonic fibroblasts derived from *Cd/Cd* mice responds to Wnt3a but Wnt signaling is not antagonized by DKK1, producing a net gain of function effect (85). This mutation provides a strong indication that canonical Wnt signaling has a role in neurulation in addition to the non-canonical Wnt pathway. Intracellular folate levels in *Cd* mice are normal and added folic acid in their diet increases intracellular levels, indicating fully functional folate transport. However, gene expression array patterns of *Cd/Cd* mutants maintained on a high folic acid diet cluster with those of wild type siblings receiving a no-folate diet, so that the *Lrp6*^{Cd} mutation is associated with a defect in intracellular folate utilization (86). Our recent studies (Gray et al., unpublished) indicate that *Lrp6*^{-/-} null mice are also sensitive to dietary folic acid supplementation, demonstrating that the folate metabolic pathway interacts with LRP6 function and the effect is not idiosyncratic to the *Lrp6*^{Cd} allele. It will be critically important to determine whether folic acid exerts its effects on canonical, PCP, or both aspects of Wnt signaling pathways and whether the primary contribution to Wnt signaling is on cell proliferation or methylation-dependent epigenetic effects.

There is also potential for folic acid supplementation to compensate for some NTD-associated drug exposures. Interestingly, folic acid supplementation in CD1 mice can reduce the occurrence of NTDs precipitated by a retinoic acid competitive antagonist (87). In addition, a recent study found that as little as 4-week treatment with retinoids for acne can reduce plasma folate concentrations in adult patients (88), suggesting possible interaction between retinoid and folate metabolic pathways. It is unclear from either clinical or experimental animal data whether maternal folic acid supplementation can prevent NTD due to VPA or other AED administration (50,89–91). The most compelling population data to date found no protection against AED-associated NTDs when folic acid is added to medication regimens (91). If the major teratogenic effect of VPA is indeed through its HDACi function, then folic acid supplementation effects on DNA methylation might have variable ability to counter VPA-induced chromatin remodeling by altered histone acetylation, depending on timing of administration, dose, and individual genetic background. Resolution of these issues and improved strategies for countering AED teratogenicity must await understanding of how specific drugs promote NTD in individuals.

Conclusion

Decades of investigation have yielded a diverse collection of genetic associations with NTDs in the mouse, epidemiological evidence for a few gene associations in human populations, and identification of environmental exposures and health conditions that increase NTD risk. Accumulating evidence indicates that no single factor ensures that neurulation will fail, but that polymorphisms in several genes, and/or coupled with the *in utero* environment, must collaborate to result in an NTD. It is now becoming feasible to take a systems approach to integrating these influences into profiles that may permit assessment of an individual couple's risk for having a child with an NTD, and to predict the preventative measure most likely to promote a healthy birth outcome. Realizing this potential will require application of high through-put technologies for population based genomics, combined with new computational tools for identifying rare variant SNPs and evaluating gene-gene interactions in a network context rather than one gene at a time. Putative interactions will have to be validated in biological models. It will be important to understand metabolic pathway 'mechanics' or homeostasis in an individual and how that will impact gene network function. Because the overt NTD phenotypes are readily recognized in humans and experimental animals, NTDs may

well be the first complex genetic disorder for which gene-gene and gene-environment interactions can be understood in depth. Progress made for this disorder can provide useful analytical tools for identifying molecular network interactions relevant to later-onset complex genetic disorders, like schizophrenia and autism.

References

1. Gelineau-van Waes J, Finnell RH. Genetics of neural tube defects. *Semin Pediatr Neurol* 2001;8:160–4. [PubMed: 11575845]
2. Copp AJ, Greene ND, Murdoch JN. The genetic basis of mammalian neurulation. *Nat Rev Genet* 2003;4:784–93. [PubMed: 13679871]
3. Harris MJ. Insights into prevention of human neural tube defects by folic acid arising from consideration of mouse mutants. *Birth Defects Res A Clin Mol Teratol* 2009;85:331–9. [PubMed: 19117321]
4. Gray JD, Ross ME. Mechanistic insights into folate supplementation from Crooked tail and other NTD-prone mutant mice. *Birth Defects Res A Clin Mol Teratol* 2009;85:314–21. [PubMed: 19067399]
5. Harris MJ, Juriloff DM. Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects Res A Clin Mol Teratol* 2007;79:187–210. [PubMed: 17177317]
6. Wald N, Sneddon J, Densem J, Frost C, Stone R, MRC VSRG. Prevention of neural tube defects: results of the medical research council vitamin study. *Lancet* 1991;338:131–137. [PubMed: 1677062]
7. Czeizel AE, Toth M, Rockenbauer M. Population-based case control study of folic acid supplementation during pregnancy. *Teratology* 1996;53:345–51. [PubMed: 8910980]
8. Shaw GM, Schaffer D, Velie EM, Morland K, Harris JA. Periconceptional vitamin use, dietary folate, and the occurrence of neural tube defects. *Epidemiology* 1995;6:219–26. [PubMed: 7619926]
9. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* 1999;341:1485–90. [PubMed: 10559448]
10. Blom HJ, Shaw GM, den Heijer M, Finnell RH. Neural tube defects and folate: case far from closed. *Nat Rev Neurosci* 2006;7:724–31. [PubMed: 16924261]
11. Beaudin AE, Stover PJ. Folate-mediated one-carbon metabolism and neural tube defects: balancing genome synthesis and gene expression. *Birth Defects Res C Embryo Today* 2007;81:183–203. [PubMed: 17963270]
12. Miller AL. The methylation, neurotransmitter, and antioxidant connections between folate and depression. *Altern Med Rev* 2008;13:216–26. [PubMed: 18950248]
13. Holmes LB, Driscoll SG, Atkins L. Etiologic heterogeneity of neural-tube defects. *N Engl J Med* 1976;294:365–9. [PubMed: 1107843]
14. Janerich DT, Piper J. Shifting genetic patterns in anencephaly and spina bifida. *J Med Genet* 1978;15:101–5. [PubMed: 641940]
15. Elwood, JM.; Little, J.; Elwood, JH. *Epidemiology and Control of Neural Tube Defects*. Oxford University Press; Oxford: 1992.
16. Shaw GM, Jensvold NG, Wasserman CR, Lammer EJ. Epidemiologic characteristics of phenotypically distinct neural tube defects among 0.7 million California births, 1983–1987. *Teratology* 1994;49:143–9. [PubMed: 8016745]
17. Neumann PE, Frankel WN, Letts VA, Coffin JM, Copp AJ, Bernfield M. Multifactorial inheritance of neural tube defects: localization of the major gene and recognition of modifiers in ct mutant mice. *Nat Genet* 1994;6:357–62. [PubMed: 8054974]
18. Gustavsson P, Greene ND, Lad D, Pauws E, de Castro SC, Stanier P, Copp AJ. Increased expression of Grainyhead-like-3 rescues spina bifida in a folate-resistant mouse model. *Hum Mol Genet* 2007;16:2640–6. [PubMed: 17720888]
19. Juriloff DM, Gunn TM, Harris MJ, Mah DG, Wu MK, Dewell SL. Multifactorial genetics of exencephaly in SELH/Bc mice. *Teratology* 2001;64:189–200. [PubMed: 11598925]

20. Kim TH, Goodman J, Anderson KV, Niswander L. Phactr4 regulates neural tube and optic fissure closure by controlling PP1-, Rb-, and E2F1-regulated cell-cycle progression. *Dev Cell* 2007;13:87–102. [PubMed: 17609112]
21. Curtin JA, Quint E, Tshipouri V, Arkell RM, Cattanach B, Copp AJ, Henderson DJ, Spurr N, Stanier P, Fisher EM, et al. Mutation of *Celsr1* disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr Biol* 2003;13:1129–33. [PubMed: 12842012]
22. Carroll EA, Gerrelli D, Gasca S, Berg E, Beier DR, Copp AJ, Klingensmith J. Cordon-bleu is a conserved gene involved in neural tube formation. *Dev Biol* 2003;262:16–31. [PubMed: 14512015]
23. Ybot-Gonzalez P, Savery D, Gerrelli D, Signore M, Mitchell CE, Faux CH, Greene ND, Copp AJ. Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development* 2007;134:789–99. [PubMed: 17229766]
24. Wang J, Hamblet NS, Mark S, Dickinson ME, Brinkman BC, Segil N, Fraser SE, Chen P, Wallingford JB, Wynshaw-Boris A. Dishevelled genes mediate a conserved mammalian PCP pathway to regulate convergent extension during neurulation. *Development* 2006;133:1767–78. [PubMed: 16571627]
25. Wang Y, Guo N, Nathans J. The role of *Frizzled3* and *Frizzled6* in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J Neurosci* 2006;26:2147–56. [PubMed: 16495441]
26. Montcouquiol M, Sans N, Huss D, Kach J, Dickman JD, Forge A, Rachel RA, Copeland NG, Jenkins NA, Bogani D, et al. Asymmetric localization of *Vangl2* and *Fz3* indicate novel mechanisms for planar cell polarity in mammals. *J Neurosci* 2006;26:5265–75. [PubMed: 16687519]
27. Park TJ, Haigo SL, Wallingford JB. Ciliogenesis defects in embryos lacking *inturned* or *fuzzy* function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat Genet* 2006;38:303–11. [PubMed: 16493421]
28. Kibar Z, Torban E, McDearmid JR, Reynolds A, Berghout J, Mathieu M, Kirillova I, De Marco P, Merello E, Hayes JM, et al. Mutations in *VANGL1* associated with neural-tube defects. *N Engl J Med* 2007;356:1432–7. [PubMed: 17409324]
29. Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* 1997;277:1109–13. [PubMed: 9262482]
30. Hui CC, Joyner AL. A mouse model of greig cephalopolysyndactyly syndrome: the extra-toes1 mutation contains an intragenic deletion of the *Gli3* gene. *Nat Genet* 1993;3:241–6. [PubMed: 8387379]
31. Liu A, Wang B, Niswander LA. Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors. *Development* 2005;132:3103–11. [PubMed: 15930098]
32. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, Leitch CC, Chapple JP, Munro PM, Fisher S, et al. Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet* 2005;37:1135–40. [PubMed: 16170314]
33. Bach A, Lallemand Y, Nicola MA, Ramos C, Mathis L, Maufras M, Robert B. *Msx1* is required for dorsal diencephalon patterning. *Development* 2003;130:4025–36. [PubMed: 12874124]
34. Ishii M, Han J, Yen HY, Sucov HM, Chai Y, Maxson RE Jr. Combined deficiencies of *Msx1* and *Msx2* cause impaired patterning and survival of the cranial neural crest. *Development* 2005;132:4937–50. [PubMed: 16221730]
35. Lallemand Y, Bensoussan V, Cloment CS, Robert B. *Msx* genes are important apoptosis effectors downstream of the *Shh/Gli3* pathway in the limb. *Dev Biol* 2009;331:189–98. [PubMed: 19422820]
36. Zhu H, Kartiko S, Finnell RH. Importance of gene-environment interactions in the etiology of selected birth defects. *Clin Genet* 2009;75:409–23. [PubMed: 19459879]
37. Eriksson UJ, Cederberg J, Wentzel P. Congenital malformations in offspring of diabetic mothers--animal and human studies. *Rev Endocr Metab Disord* 2003;4:79–93. [PubMed: 12618562]
38. Werler MM, Louik C, Shapiro S, Mitchell AA. Prepregnant weight in relation to risk of neural tube defects. *Jama* 1996;275:1089–92. [PubMed: 8601927]
39. Hendricks KA, Nuno OM, Suarez L, Larsen R. Effects of hyperinsulinemia and obesity on risk of neural tube defects among Mexican Americans. *Epidemiology* 2001;12:630–5. [PubMed: 11679789]
40. Shaw GM, Quach T, Nelson V, Carmichael SL, Schaffer DM, Selvin S, Yang W. Neural tube defects associated with maternal periconceptional dietary intake of simple sugars and glycemic index. *Am J Clin Nutr* 2003;78:972–8. [PubMed: 14594784]

41. Wentzel P, Wentzel CR, Gareskog MB, Eriksson UJ. Induction of embryonic dysmorphogenesis by high glucose concentration, disturbed inositol metabolism, and inhibited protein kinase C activity. *Teratology* 2001;63:193–201. [PubMed: 11320530]
42. Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW Jr, Lott IT, et al. Retinoic acid embryopathy. *N Engl J Med* 1985;313:837–41. [PubMed: 3162101]
43. Azais-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits. *Am J Clin Nutr* 2000;71:1325S–33S. [PubMed: 10799410]
44. Rosa FW, Wilk AL, Kelsey FO. Teratogen update: vitamin A congeners. *Teratology* 1986;33:355–64. [PubMed: 3461576]
45. Kapron-Bras CM, Trasler DG. Reduction in the frequency of neural tube defects in splotch mice by retinoic acid. *Teratology* 1985;32:87–92. [PubMed: 3898457]
46. Chen WH, Morriss-Kay GM, Copp AJ. Genesis and prevention of spinal neural tube defects in the curly tail mutant mouse: involvement of retinoic acid and its nuclear receptors RAR-beta and RAR-gamma. *Development* 1995;121:681–91. [PubMed: 7720576]
47. Liu J, Qi J, Zhu J, Zhang L, Liang Y, Ning Q, Luo X. Effects of Retinoic Acid on the Expressions of Vangl1 and Vangl2 in Mouse Fetuses. *J Neurogenet* 2008;1–12. [PubMed: 19012162]
48. Ornoy A. Neuroteratogens in man: an overview with special emphasis on the teratogenicity of antiepileptic drugs in pregnancy. *Reprod Toxicol* 2006;22:214–26. [PubMed: 16621443]
49. Dansky LV, Finnell RH. Parental epilepsy, anticonvulsant drugs, and reproductive outcome: epidemiologic and experimental findings spanning three decades; 2: Human studies. *Reprod Toxicol* 1991;5:301–35. [PubMed: 1806139]
50. Duncan S, Mercho S, Lopes-Cendes I, Seni MH, Benjamin A, Dubeau F, Andermann F, Andermann E. Repeated neural tube defects and valproate monotherapy suggest a pharmacogenetic abnormality. *Epilepsia* 2001;42:750–3. [PubMed: 11422330]
51. Defoort EN, Kim PM, Winn LM. Valproic acid increases conservative homologous recombination frequency and reactive oxygen species formation: a potential mechanism for valproic acid-induced neural tube defects. *Mol Pharmacol* 2006;69:1304–10. [PubMed: 16377765]
52. Faiella A, Wernig M, Consalez GG, Hostick U, Hofmann C, Hustert E, Boncinelli E, Balling R, Nadeau JH. A mouse model for valproate teratogenicity: parental effects, homeotic transformations, and altered HOX expression. *Hum Mol Genet* 2000;9:227–36. [PubMed: 10607833]
53. Okada A, Kushima K, Aoki Y, Bialer M, Fujiwara M. Identification of early-responsive genes correlated to valproic acid-induced neural tube defects in mice. *Birth Defects Res A Clin Mol Teratol* 2005;73:229–38. [PubMed: 15799026]
54. Stodgell CJ, Ingram JL, O'Bara M, Tisdale BK, Nau H, Rodier PM. Induction of the homeotic gene *Hoxa1* through valproic acid's teratogenic mechanism of action. *Neurotoxicol Teratol* 2006;28:617–24. [PubMed: 16989981]
55. Menegola E, Di Renzo F, Broccia ML, Prudenziati M, Minucci S, Massa V, Giavini E. Inhibition of histone deacetylase activity on specific embryonic tissues as a new mechanism for teratogenicity. *Birth Defects Res B Dev Reprod Toxicol* 2005;74:392–8. [PubMed: 16193500]
56. Milutinovic S, D'Alessio AC, Detich N, Szyf M. Valproate induces widespread epigenetic reprogramming which involves demethylation of specific genes. *Carcinogenesis* 2007;28:560–71. [PubMed: 17012225]
57. Smithells RW, Sheppard S, Schorah CJ. Vitamin deficiencies and neural tube defects. *Arch Dis Child* 1976;51:944–50. [PubMed: 1015847]
58. Smithells RW, Nevin NC, Seller MJ, Sheppard S, Harris R, Read AP, Fielding DW, Walker S, Schorah CJ, Wild J. Further experience of vitamin supplementation for prevention of neural tube defect recurrences. *Lancet* 1983;1:1027–31. [PubMed: 6133069]
59. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832–5. [PubMed: 1307234]
60. Beaudin AE, Stover PJ. Insights into metabolic mechanisms underlying folate-responsive neural tube defects: a minireview. *Birth Defects Res A Clin Mol Teratol* 2009;85:274–84. [PubMed: 19180567]
61. Piedrahita JA, Oetama B, Bennett GD, van Waes J, Kamen BA, Richardson J, Lacey SW, Anderson RG, Finnell RH. Mice lacking the folic acid-binding protein *Folbp1* are defective in early embryonic development. *Nat Genet* 1999;23:228–32. [PubMed: 10508523]

62. Gelineau-van Waes J, Heller S, Bauer LK, Wilberding J, Maddox JR, Aleman F, Rosenquist TH, Finnell RH. Embryonic development in the reduced folate carrier knockout mouse is modulated by maternal folate supplementation. *Birth Defects Res A Clin Mol Teratol* 2008;82:494–507. [PubMed: 18383508]
63. Rothenberg SP, da Costa MP, Sequeira JM, Cracco J, Roberts JL, Weedon J, Quadros EV. Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect. *N Engl J Med* 2004;350:134–42. [PubMed: 14711912]
64. Molloy AM, Quadros EV, Sequeira JM, Troendle JF, Scott JM, Kirke PN, Mills JL. Lack of association between folate-receptor autoantibodies and neural-tube defects. *N Engl J Med* 2009;361:152–60. [PubMed: 19587340]
65. Cabrera RM, Shaw GM, Ballard JL, Carmichael SL, Yang W, Lammer EJ, Finnell RH. Autoantibodies to folate receptor during pregnancy and neural tube defect risk. *J Reprod Immunol* 2008;79:85–92. [PubMed: 18804286]
66. Shaw GM, Lammer EJ, Zhu H, Baker MW, Neri E, Finnell RH. Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G), and risk of spina bifida. *Am J Med Genet* 2002;108:1–6. [PubMed: 11857541]
67. Heid MK, Bills ND, Hinrichs SH, Clifford AJ. Folate deficiency alone does not produce neural tube defects in mice. *J Nutr* 1992;122:888–94. [PubMed: 1552363]
68. Burgoon JM, Selhub J, Nadeau M, Sadler TW. Investigation of the effects of folate deficiency on embryonic development through the establishment of a folate deficient mouse model. *Teratology* 2002;65:219–27. [PubMed: 11967921]
69. Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, Chen MF, Pai A, John SW, Smith RS, et al. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. *Hum Mol Genet* 2001;10:433–43. [PubMed: 11181567]
70. Molloy AM, Kirke P, Hillary I, Weir DG, Scott JM. Maternal serum folate and vitamin B12 concentrations in pregnancies associated with neural tube defects. *Arch Dis Child* 1985;60:660–5. [PubMed: 4026363]
71. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Q J Med* 1993;86:703–8. [PubMed: 8265769]
72. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am J Epidemiol* 2000;151:862–77. [PubMed: 10791559]
73. Davalos IP, Olivares N, Castillo MT, Cantu JM, Ibarra B, Sandoval L, Moran MC, Gallegos MP, Chakraborty R, Rivas F. The C677T polymorphism of the methylenetetrahydrofolate reductase gene in Mexican mestizo neural-tube defect parents, control mestizo and native populations. *Ann Genet* 2000;43:89–92. [PubMed: 10998450]
74. Boyles AL, Billups AV, Deak KL, Siegel DG, Mehlretter L, Slifer SH, Bassuk AG, Kessler JA, Reed MC, Nijhout HF, et al. Neural tube defects and folate pathway genes: family-based association tests of gene-gene and gene-environment interactions. *Environ Health Perspect* 2006;114:1547–52. [PubMed: 17035141]
75. Castro R, Rivera I, Ravasco P, Camilo ME, Jakobs C, Blom HJ, de Almeida IT. 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C-->T and 1298A-->C mutations are associated with DNA hypomethylation. *J Med Genet* 2004;41:454–8. [PubMed: 15173232]
76. Sawabe K, Wakasugi KO, Hasegawa H. Tetrahydrobiopterin uptake in supplemental administration: elevation of tissue tetrahydrobiopterin in mice following uptake of the exogenously oxidized product 7,8-dihydrobiopterin and subsequent reduction by an anti-folate-sensitive process. *J Pharmacol Sci* 2004;96:124–33. [PubMed: 15467264]
77. Nachmany A, Gold V, Tsur A, Arad D, Weil M. Neural tube closure depends on nitric oxide synthase activity. *J Neurochem* 2006;96:247–53. [PubMed: 16300634]
78. Bottiglieri T. Homocysteine and folate metabolism in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;29:1103–12. [PubMed: 16109454]
79. Moretti P, Sahoo T, Hyland K, Bottiglieri T, Peters S, del Gaudio D, Roa B, Curry S, Zhu H, Finnell RH, et al. Cerebral folate deficiency with developmental delay, autism, and response to folinic acid. *Neurology* 2005;64:1088–90. [PubMed: 15781839]

80. Kent C, Carman GM. Interactions among pathways for phosphatidylcholine metabolism, CTP synthesis and secretion through the Golgi apparatus. *Trends Biochem Sci* 1999;24:146–50. [PubMed: 10322420]
81. Fleming A, Copp AJ. Embryonic folate metabolism and mouse neural tube defects. *Science* 1998;280:2107–9. [PubMed: 9641914]
82. Wlodarczyk BJ, Tang LS, Triplett A, Aleman F, Finnell RH. Spontaneous neural tube defects in splotch mice supplemented with selected micronutrients. *Toxicol Appl Pharmacol* 2006;213:55–63. [PubMed: 16226775]
83. Burren KA, Savery D, Massa V, Kok RM, Scott JM, Blom HJ, Copp AJ, Greene ND. Gene-environment interactions in the causation of neural tube defects: folate deficiency increases susceptibility conferred by loss of Pax3 function. *Hum Mol Genet*. 2008
84. Carter M, Ulrich S, Oofuji Y, Williams DA, Ross ME. Crooked tail (Cd) models human folate-responsive neural tube defects. *Hum Mol Genet* 1999;8:2199–204. [PubMed: 10545599]
85. Carter M, Chen X, Slowinska B, Minnerath S, Glickstein S, Shi L, Campagne F, Weinstein H, Ross ME. Crooked tail (Cd) model of human folate-responsive neural tube defects is mutated in Wnt coreceptor lipoprotein receptor-related protein 6. *Proc Natl Acad Sci U S A* 2005;102:12843–8. [PubMed: 16126904]
86. Ernest S, Carter M, Shao H, Hosack A, Lerner N, Colmenares C, Rosenblatt DS, Pao YH, Ross ME, Nadeau JH. Parallel changes in metabolite and expression profiles in crooked-tail mutant and folate-reduced wild-type mice. *Hum Mol Genet* 2006;15:3387–93. [PubMed: 17050573]
87. Cipollone D, Carsetti R, Tagliani A, Rosado MM, Borgiani P, Novelli G, D'Amati G, Fumagalli L, Marino B, Businaro R. Folic acid and methionine in the prevention of teratogen-induced congenital defects in mice. *Cardiovasc Pathol* 2009;18:100–9. [PubMed: 18417366]
88. Chanson A, Cardinault N, Rock E, Martin JF, Souteyrand P, D'Incan M, Brachet P. Decreased plasma folate concentration in young and elderly healthy subjects after a short-term supplementation with isotretinoin. *J Eur Acad Dermatol Venereol* 2008;22:94–100. [PubMed: 18181979]
89. Craig J, Morrison P, Morrow J, Patterson V. Failure of periconceptual folic acid to prevent a neural tube defect in the offspring of a mother taking sodium valproate. *Seizure* 1999;8:253–4. [PubMed: 10452927]
90. Yerby MS. Management issues for women with epilepsy: neural tube defects and folic acid supplementation. *Neurology* 2003;61:S23–6. [PubMed: 14504306]
91. Morrow JI, Hunt SJ, Russell AJ, Smithson WH, Parsons L, Robertson I, Waddell R, Irwin B, Morrison PJ, Craig JJ. Folic acid use and major congenital malformations in offspring of women with epilepsy: a prospective study from the UK Epilepsy and Pregnancy Register. *J Neurol Neurosurg Psychiatry* 2009;80:506–11. [PubMed: 18977812]

Figure 1.

Folate metabolism and its contributions to physiological processes. One carbon metabolism in the cytoplasm is depicted. Colors: Blue=enzymes catalyzing folate metabolic steps; Red=important products of the pathway; Magenta=physiological processes which pathway products support. THF=tetrahydrofolate; DHF=dihydrofolate; dUMP=deoxyuridine-monophosphate; Ado-Met=S-adenosylmethionine; Ado-Hcy=S-adenosylhomocysteine; BH₄=tetrahydrobiopterin; qBH₂=quinonoid dihydrobiopterin; PtdMME=phosphatidylethanolamine (monomethyl); PtdDME=phosphatidylethanolamine (dimethyl); PEMT=phosphatidylethanolamine methyltransferase. FTHFS=10-formyltetrahydrofolate synthase; MTHFC=methyltetrahydrofolate cyclohydrolase; MTHFD=methyltetrahydrofolate dehydrogenase; MTHFR=methyltetrahydrofolate reductase; MS=methionine synthase; cSHMT=cytoplasmic serine hydroxymethyltransferase; DHFR=dihydrofolate reductase; TS=thymidylate synthase