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

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SUMMARY

Neural tube defects (NTDs) may be associated with syndromes, disorders, and maternal and fetal risk factors. This article provides a comprehensive review of the syndromes, disorders, and maternal and fetal risk factors associated with NTDs, including maternal fumonisin consumption, periconceptional zinc deficiency, parental occupational exposure and residential proximity to pesticides, lower socioeconomic status, fetal alcohol syndrome, mutations in the *VANGL1* gene, human athymic Nude/SCID fetus, and single nucleotide polymorphism in the *NOS3* gene. NTDs associated with these syndromes, disorders, and maternal and fetal risk factors are a rare but important cause of NTDs. The recurrence risk and the preventive effect of maternal folic acid intake in NTDs associated with syndromes, disorders and maternal risk factors may be different from those of nonsyndromic multifactorial NTDs. Perinatal diagnosis of NTDs should alert doctors to the syndromes, disorders, and maternal and fetal risk factors associated with NTDs, and prompt thorough etiologic investigation and genetic counseling. [*Taiwan J Obstet Gynecol* 2008;47(3):267–275]

Key Words: congenital malformations, disorder, maternal risk factors, neural tube defects, syndromes

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, while less common types include amniotic band syndrome, limb–body wall complex, cloacal exstrophy or omphalocele-exstrophy-imperforate anus-spinal defects complex and other types of spinal abnormalities. The incidence of NTDs varies with race, geographic location, socioeconomic class, 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 controlling folate metabolism. There is considerable evidence to suggest that genetic and environmental factors contribute to the etiology of NTDs. NTDs may be associated with maternal and fetal risk factors.

Maternal Fumonisin Consumption

Fumonisins are a family of mycotoxins produced by the fungus *Fusarium verticillioides*, which can infect corns and cause diseases of corn ears, stalks and seedlings [1]. Unintended consumption of contaminated corns or corn products may result in cancer, diseases, and congenital anomalies in humans and animals [2]. Fumonisin consumption is a risk factor for human NTDs, especially in combination with other risk factors such as genetic susceptibility and folate deficiency [2]. A relationship

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Accepted: June 26, 2008

between maternal fumonisin consumption and NTDs is most evident among populations that consume the highest amounts of corns, such as those of Texas–Mexico border, Central and South America, South Africa, and northern provinces of China [3–8]. Hendricks [5] found that corn meal samples collected in the United States during the NTD outbreak between 1990 and 1991 had a relatively high level of fumonisins. Missmer et al [8] found that during the NTD outbreak along the Texas–Mexico border between 1990 and 1991, moderate (301–400 tortillas) periconceptional corn tortilla intake during the first trimester was associated with increased odds ratios (ORs) of having an NTD-affected pregnancy (OR, 2.4; 95% confidence interval [CI], 1.1–5.3) compared with low (≤ 100 tortillas) periconceptional consumption, and no increased risks were observed at high (≥ 400 tortillas) periconceptional consumption. Missmer et al [8] suggested that maternal fumonisin consumption increases the risk of NTD, and the risk is proportional to dose up to a threshold level at which point fetal death may occur. Fumonisins induce NTDs by their apparent interference with folate utilization. Fumonisins inhibit sphingolipid biosynthesis by the inhibition of ceramide synthase [9]. The fumonisin-induced sphingolipid deletion affects folate receptor-mediated transport of 5-methyltetrahydrofolate and results in folate deficiency and NTDs [10]. In mouse models, fumonisin exposure induces NTDs [11–13], and supplemental folic acid prevents fumonisin-induced NTDs [12,13].

Periconceptional Zinc Deficiency

Zinc is necessary for fetal growth and development. It is a constituent of hormones and neuropeptides [14], and is essential for cell proliferation and differentiation [15]. Neonates born with NTDs have been found to have low serum and/or hair zinc levels [16–19]. In animal models, severely reduced maternal zinc intake in early gestation results in NTDs in offspring [20]. In humans, zinc deficiency is related to central nervous system malformations [21–25]. Sever [21] observed that chronic low dietary zinc intake in Egypt, the Middle East, and among Sikhs was associated with increased prevalence of NTDs. Pregnant women with acrodermatitis enteropathica are at risk for NTDs [22]. Acrodermatitis enteropathica (OMIM 201100) is an autosomal recessive disorder caused by mutation in the *SLC39A4* (OMIM 607059) gene. The *SLC39A4* gene encodes the zinc transporter protein ZIP4, which is essential for normal zinc homeostasis [26–31]. Acrodermatitis enteropathica is a disorder of zinc deficiency due to impaired absorption of zinc from the gastrointestinal tract. The clinical features of acrodermatitis enteropathica include alopecia, diarrhea, vascular dermatitis, dysfunction of humoral and cellular immunity, and failure to thrive. Acrodermatitis enteropathica can be treated by oral zinc supplementation with 3 mg/kg/day of elemental zinc [32]. In the case of acrodermatitis enteropathica, the amount of zinc supplementation may need to be increased during pregnancy [33]. This is because pregnancy as well as oral contraceptive use can cause a decrease in zinc concentration, possibly due to the effect of estrogen [34–36]. Furthermore, prenatal iron supplementation can impair zinc absorption [37,38]. Vegetarians, alcoholics and the malnourished are at increased risk of zinc deficiency, because the bioavailability of zinc in vegetarian diet with abundant phylate is low, and alcoholics usually have malnutrition, impaired zinc absorption and increased urinary excretion of zinc [32]. Following zinc supplementation, the pregnancy outcome in women with acrodermatitis enteropathica is good [39]. Zinc supplementation is also beneficial in reducing the NTD risk in pregnant women with chronic zinc deficiency and recurrent NTD births [40–42]. Velie et al [43] proposed that the risk of NTDs in fetuses and infants decreases with increasing maternal periconceptional zinc intake, and inadequate maternal zinc intake is associated with increased NTD risk. They observed that: (1) increased maternal periconceptional zinc intake from all sources and from the most bioavailable source such as animal products was associated with decreased NTD risk; (2) low total zinc intake in combination with high phytate intake was associated with the highest risk for NTDs; (3) and the association between total zinc intake and NTD risk was not mediated by total folate intake or by sociodemographic factors. Groenen et al [44,45] concluded that maternal zinc status is associated with the risk of spina bifida in offspring. Groenen et al [44] found that the maternal red blood cell zinc concentration was significantly lower at 5% (95% CI, 0–9%) in mothers with NTD offspring compared with the control mothers, and the OR for maternal low zinc concentration in association with spina bifida was 2.9 (95% CI, 1.2–7.0). Cengiz et al [46] found that in women with second-trimester induced abortion resulting from NTDs had significantly lower serum zinc level (62.48 ± 15.9 *vs*. 102.6 ± 23.7), and suggested zinc supplementation in addition to folic acid supplementation to decrease the recurrence and occurrence of NTDs. Bound et al [47] suggested that lead may induce NTDs by reducing bioavailability of zinc from food and indirectly causing zinc deficiency. However, the suggestion is contrary to the results of three studies: (1) Macdonell et al [48] found no correlation between domestic water lead concentration and

the prevalence of NTDs; (2) Carrillo-Ponce Mde et al [18] found no correlation between NTDs and a high level of serum lead in newborns; (3) and Brender et al [49] found no significant difference in blood level of lead between women with NTD-affected pregnancies and women with normal live births. Brender et al [49] suggested that maternal exposure to lead is probably not a significant risk factor for NTDs in offspring.

Parental Occupational Exposure and Residential Proximity to Pesticides

Parental occupational exposure and residential proximity to teratogens have been associated with an increased risk of NTDs in offspring. There is sufficient evidence of the adverse reproductive effects of NTDs in maternal or paternal exposure to pesticides and/or agricultural work [50–61]. High-level pesticide exposure may occur in pesticide applicators, manufacturers and other agricultural workers, and moderate- and low-level exposures may occur in the general populations from residential pesticide use or dietary contamination. In a study in England on congenital malformations among offspring of agricultural workers, Balarajan and McDowall [50] observed that gardeners and groundsmen who used herbicide experienced increased ratios for spina bifida, anencephaly and facial clefts. In a study in the Netherlands, Blatter et al [51] found an increased risk of spina bifida in women working in agricultural occupations (OR, 3.4; 95% CI, 1.3–9.0). In another study also in the Netherlands, Blatter et al [52] found an OR of 5.6 (95% CI, 1.8–17.81) for maternal agricultural occupations. Blatter and Roeleveld [53] found an increased risk of spina bifida in women working in agricultural occupations in Sweden (OR, 1.8; 95% CI, 0.8–4.2). Blatter et al [54] found an association between spina bifida and paternal pesticide occupational exposure (OR, 1.7; 95% CI, 0.7–4.0). Blatter et al [55] observed increased ORs for women in agricultural occupations in Sweden (OR, 1.8; 95% CI, 0.8–4.2) and in Spain (OR, 2.2; 95% CI, 0.8–5.9) but not in Hungary (OR, 1.1; 95% CI, 0.7–1.7). In Spain, García et al [56] found that the mothers who were involved in agricultural activities during the month before conception and the first trimester of pregnancy had an OR of 3.16 (95% CI, 1.11–9.01) for the risk of nervous system defects, oral clefts and multiple anomalies; paternal agricultural work did not increase the risk except that the fathers, who reported ever handling pesticides, had an OR of 1.49 (95% CI, 0.94–2.35) for nervous system and musculoskeletal defects. In California, Shaw et al [57] found that women living within 0.25 miles of an agricultural crop had increased risks for offspring with NTDs (OR, 1.5; 95% CI, 1.1–2.1), and Shaw et al [58] also found that fathers who worked in farming, forestry and fishing had an OR of 2.1 (95% CI, 1.3–3.3) for NTDs. In Norway, Nordby et al [59] found that NTDs was moderately associated with potato cultivation (prevalence ratio [PR], 1.6; 95% CI, 1.1–2.3) and paternal work of > 500 hours/year (PR, 1.6; 95% CI, 1.1–2.5), and suggested that there is a moderate association of exposure to mancozeb, a fungicide, with NTDs. In California, Rull et al [60] observed that increases in NTD risks were associated with maternal residence within 1,000 m of agricultural applications of benomyl, methyl carbamate or organophosphorus pesticides, or pesticides listed as endocrine disruptors, cholinesterase inhibitors or developmental toxins. They also found that cholinesterase inhibitors and organophosphorus pesticides were specific for anencephaly, and amides, benzimidazoles and methyl carbamates were specific for spina bifida. Lacasaña et al [61] suggested that maternal exposure to agricultural work is a risk factor for NTDs, and paternal exposure to pesticides in or prior to periconceptional period increases the risk of having an anencephalic child. In Mexico, Lacasaña et al [61] found that the children of mothers who worked in agriculture in the acute risk period of 3 months before and 1 month after the last menstruation periods had a greater risk of anencephaly (OR, 4.57; 95% CI, 1.05–19.96), and the risk of fathers having a child with anencephaly was greater in those who applied pesticides either in the acute risk period (OR, 2.50; 95% CI, 0.73–8.64) or in the non-acute risk period (OR, 2.03; 95% CI, 0.58–7.08). Lacasaña et al [61] advised that women, who are involved in agricultural work or living with men working in agriculture, should be protected from direct and indirect pesticide exposure, especially during the periconceptional period if they are planning to have a child.

Lower Socioeconomic Status

NTDs have been reported in populations with lower socioeconomic status (SES) as measured by education, occupation and income [62–66]. Education reflects a person's ability to access and interpret health-related information; occupation influences health via exposure to work place hazards, psychosocial factors, and social network; and income influences maternal health, living condition, medical care, and lifestyle [62]. Wasserman et al [63] observed twofold elevated NTD risks for several SES indicators and suggested that both lower SES and residence in a lower-SES neighborhood

increase the risk of an NTD-affected pregnancy. Farley et al [64] found that low maternal education was a strong predictor of having a child with an NTD (adjusted OR [AOR], 1.8; 95% CI, 1.1–3.1) and suggested that interventions should target women of low educational status in order to further reduce the incidence of NTDs. Meyer and Siega-Riz [65] observed that the decline in spina bifida from 1995 to 1999 in North Carolina after folic acid fortification varied considerably by sociodemographic subpopulations. In a Mexican study, Blanco Muñoz et al [66] identified four groups of women that may be especially vulnerable to NTDs, which included women with less than a primary school education (AOR, 3.0; 95% CI, 1.2–7.6), women with a primary school education but without a completed junior high school education (AOR, 2.2; 95% CI, 0.9–5.7), women with a monthly income < US$100 (AOR, 2.5; 95% CI, 1.2–5.1), and women employed in industry or agriculture during the acute period of 3 months prior to conception to 1 month after conception (AOR, 6.5; 95% CI, 1.4–29.6). In a large, multicentered, US case-control study, Yang et al [62] found that maternal low education was associated with anencephaly (AOR, 1.4; 95% CI, 0.6–3.4), paternal operator/laborer occupation was associated with spina bifida (AOR, 1.4; 95% CI, 1.0–2.0), and subjects with the lowest household SES index had the greatest risk of anencephaly and spina bifida (AOR, 2.3; 95% CI, 1.0–5.5).

Fetal Alcohol Syndrome

Fetal alcohol syndrome (FAS) is characterized by prenatal and postnatal onset of growth deficiency, mild-to-moderate microcephaly, short palpebral fissures, maxillary hypoplasia, a short nose, smooth philtrum with a thin and smooth upper lip, joint anomalies, ventricular septal defects, atrial septal defects, fine motor dysfunction, irritability in infancy, hyperactivity in childhood, and varying degrees of mental retardation [67]. Most children with FAS are born to alcoholic women whose intake is eight to ten drinks or more per day [67]. Occasional abnormalities of the central nervous system associated with FAS include meningomyelocele, hydrocephalus, abnormalities of the corpus callosum, and reduced sizes of the basal ganglia and cerebellar vermis [67]. Using magnetic resonance imaging, Johnson et al [68] found central nervous system abnormalities to be associated with FAS, including agenesis and hypoplasia of the corpus callosum, cavum septi pellucidi, cavum vergae, ventriculomegaly, hypoplasia of inferior olivary eminences, small brain stem and microencephaly. A potential association between maternal early pregnancy alcohol intake and NTDs has been described [69–75]. Clarren and Smith [69] observed one child with lumbar meningomyelocele and one child with lumbosacral lipomeningocele among 65 cases of FAS. Goldstein and Arulanantham [70] reported sacral myelomeningocele and renal anomalies in a child with FAS. Clarren [71] documented two infants with anencephaly born to alcoholic mothers. Fuster et al [72] reported an infant with sacral myelomeningocele and FAS. Friedman [73] reported lumbosacral meningomyelocele in a child with maternal alcohol ingestion. Castro-Gago et al [74] reported four brothers with lumbosacral myelomeningocele, with the excessive ingestion of alcohol by their mother during the pregnancies being the only significant factor. Kan et al [75] reported an infant with sacral myelomeningocele, Arnold-Chiari malformation, and FAS. Heavy drinking during pregnancy is well known to be associated with congenital malformations. However, whether light or moderate drinking is teratogenic is controversial [76,77]. Shaw et al [78] reported that maternal alcohol use did not reveal increased NTD risks by their findings of periconceptional use of alcohol with < 1 drink/day (OR, 0.80; 95% CI, 0.62–1.0) or ≥ 1 drink/day (OR, 0.69; 95% CI, 0.4–1.2). Pathogenesis of ethanol-induced NTDs includes oxidative stress, folate deficiency, and involvement of the Sonic hedgehog (Shh) signaling. Oxidative stress and free radicals have been shown to be associated with ethanol-induced cytotoxicity in neural crest cells [79]. Ethanol-induced NTDs are caused in part by ethanol inhibition of L1-mediated cell adhesion [80]. Antioxidants that modulate the action of ethanol on the L1 cell adhesion molecules can diminish the adverse effects of ethanol [81,82]. Alcohol is also known to induce folate deficiency [83]. Ethanol impairs methionine synthase activity, alters methionine metabolism, and increases hepatocellular apoptosis and proliferation in micropigs [84]. Chronic consumption of alcohol selectively inhibits active methionine absorption in the small intestine of pregnant rats [85]. Ethanol acutely impairs the renal conservation of 5-methyltetrahydrofolate in the isolated perfused rat kidneys [86]. Chronic ethanol exposure depletes serum and hepatic folate and causes folate deficiency in rats [87]. Ethanol enhances urinary folate excretion in chronically alcoholic monkeys [88]. Yanaguita et al [89] showed that supplementation with the recommended dose of folic acid in rats was not effective in preventing the deleterious teratogenic effects induced by ethanol, indicating the need for an increased dose of folic acid in ethanol-treated mice. Shh signaling cascade is involved in the molecular pathogenesis of FAS [90,91]. Ahlgren et al [90] reported that neural crest cell death in chick embryos exposed

to excessive ethanol was associated with downregulation of Shh signaling cascade. On the contrary, Yamada et al [91] found that craniofacial anomalies in mouse embryos exposed to high ethanol were associated with increased expression of Shh signaling cascade genes.

Mutations in the *VANGL1* Gene

The *VANGL2* gene (OMIM 600533) encodes a homolog of *Drosophila* Strabismus/Van Gogh (Stbm/Vang), a highly conserved planar cell polarity protein initially involved in the frizzled-disheveled tissue polarity pathway in *Drosophila* [92]. Loop-tail (Lp) is a semidominant mouse mutation in which the *Vangl2* gene is mutated and the Lp/+ mouse heterozygotes display urogenital defects and a looped tail. Vangl2 is expressed along the neural tube at the time of closure [92]. In Lp/Lp mouse homozygotes, homozygosity for loss-of-function alleles at *Vangl2* causes craniorachischisis and *in utero* death [92]. There is a second *VANGL* gene, *VANGL1* (OMIM 610132), in vertebrates. VANGL1 and VANGL2 proteins share 73.1% similarity [93]. In humans, heterozygosity for mutant variants of *VANGL1* is associated with NTDs [94]. Kibar et al [94] identified independent mutations (V239I, R274Q and M328T) in the human *VANGL1* gene in three patients in a cohort of 144 patients representing sporadic and familial cases of NTDs. In a 10-year-old Italian girl with caudal regression syndrome, lipomyeloschisis, anorectal malformation, hydromelia and tethered spinal cord, Kibar et al [94] detected a missense mutation V239I, resulting in the substitution of valine with isoleucine in *VANGL1*. The V239I mutation was present in her mother, who showed no clinical signs of NTDs, and in her brother, who had only dermal sinus, but not in her father. In a 19-year-old Italian girl with lumbosacral myelomeningocele, hydrocephalus and congenital club feet, Kibar et al [94] detected a missense mutation R274Q, resulting in the substitution of arginine with glutamine in *VANGL1*. The R274Q mutation was present in her mother but not in her father. Her mother and maternal aunt had vertebral schisis. In a 21-year-old woman with sporadic disease of lumbosacral myelomeningocele, hydrocephalus, Chiari II malformation, tethered spinal cord, club feet, lumbosacral scoliosis and sacrococcygeal kyphosis, Kibar et al [94] detected a missense mutation M328T, resulting in the substitution of methionine with threonine in *VANGL1*. In their study, only a small proportion of NTD patients had heterozygous mutations in *VANGL1*, and there was incomplete penetrance, indicating that other environmental factors and/or sensitizing genetic lesions may also play a role in the pathogenesis of NTDs. Torban et al [95] hypothesized that partial loss of *VANGL2* function may be one of such sensitizing genetic lesions, and suggested that *VANGL1* functions in the mammalian planar cell polarity pathway and acts in concert with *VANGL2* to control neural tube formation.

Human Athymic Nude/SCID Fetus

Amorosi et al [96] reported a 15-week-gestation female human fetus homozygous for the R255X mutation in the *FOXN1* gene, who had multiple anomalies including anencephaly, spina bifida, abnormal skin and lack of thymus. Nude/SCID phenotype (OMIM 601705) is characterized by severe combined immunodeficiency (SCID) syndrome, congenital alopecia, and nail dystrophy [97]. Nude/SCID phenotype is an autosomal recessive disorder caused by homozygous mutation in the *FOXN1* gene (*WHN*; OMIM 600838) [98]. *Foxn1* is implicated in mouse cutaneous and thymic epithelial development and is associated with the mouse *nude* (*nu*) mutations [99,100]. Amorosi et al [96] found that *Foxn1* gene was expressed in the mouse developing choroid plexus cyst and suggested that FOXN1 may be involved in neurulation in humans.

Single Nucleotide Polymorphism (SNP) in the *NOS3* Gene

Nitric oxide (NO) inhibits methionine synthase activity and interferes with homocysteine remethylation [101] and proper neurulation [102–104]. Endothelial nitric oxide synthase 3 (NOS3) is expressed constitutively in endothelial cells and is responsible for vascular NO production. The single nucleotide polymorphism (SNP) *NOS3* G894T SNP (*NOS3* Glu298Asp) in the *NOS3* gene (OMIM 163729) has been shown to be associated with susceptibility to late-onset Alzheimer disease [105,106], pregnancy-induced hypertension [107], placental abruption [108,109], resistance to conventional antihypertensive therapy [110], ischemic heart disease [111], coronary spasm [112], angiographic coronary artery disease [113], ischemic stroke, [114] and NTDs [115,116]. The *NOS3* 894TT genotype is associated with plasma hyperhomocysteinemia in cases with a low serum folate concentration [117]. Brown et al [115] found that embryonic *NOS3* G894T genotype was significantly associated with the risk of spina bifida. van der Linden et al [116] found that the *NOS3* 894TT genotype did not increase spina bifida risk in mothers (OR, 1.50; 95% CI, 0.71–3.19) and in

children (OR, 1.78; 95% CI, 0.75–4.25). However, they found that maternal *NOS3* 894GT/TT genotype increased the maternal risk of having a spina bifida-affected child, in combination with either a maternal *MTHFR* 677TT genotype (OR, 4.52; 95% CI, 1.55–13.22) or an elevated plasma homocysteine (OR, 3.38; 95% CI, 1.46–7.84).

Conclusion

This article provides a comprehensive review of syndromes, disorders, and maternal and fetal risk factors associated with NTDs, including maternal fumonisin consumption, periconceptional zinc deficiency, parental occupational exposure and residential proximity to pesticides, lower socioeconomic status, FAS, mutations in the *VANGL1* gene, human athymic Nude/SCID fetus, and SNP in the *NOS3* gene. NTDs associated with syndromes, disorders, and maternal and fetal risk factors are a rare but important cause of NTDs. The recurrence risk and the preventive effect of maternal folic acid intake in NTDs associated with syndromes, disorders, and maternal and fetal risk factors may be different from those of nonsyndromic multifactorial NTDs. Perinatal diagnosis of NTDs should alert doctors to the syndromes, disorders, and maternal and fetal risk factors associated with NTDs, and prompt thorough etiologic investigation and genetic counseling.

References

1. Munkvold GP. Cultural and genetic approaches to managing mycotoxins in maize. *Annu Rev Phytopathol* 2003;41: 99–116.
2. Marasas WF, Riley RT, Hendricks KA, et al. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and *in vivo*: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *J Nutr* 2004;134: 711–6.
3. Ncayiyana DJ. Neural tube defects among rural blacks in a Transkei district. A preliminary report and analysis. *S Afr Med J* 1986;69:618–20.
4. Moore CA, Li S, Li Z, et al. Elevated rates of severe neural tube defects in a high-prevalence area in northern China. *Am J Med Genet* 1997;73:113–8.
5. Hendricks K. Fumonisins and neural tube defects in South Texas. *Epidemiology* 1999;10:198–200.
6. Hendricks KA, Simpson JS, Larsen RD. Neural tube defects along the Texas–Mexico border, 1993–1995. *Am J Epidemiol* 1999;149:1119–27.
7. Suarez L, Hendricks KA, Cooper SP, Sweeney AM, Hardy RJ, Larsen RD. Neural tube defects among Mexican Americans living on the US–Mexico border: effects of folic acid and dietary folate. *Am J Epidemiol* 2000;152:1017–123.
8. Missmer SA, Suarez L, Felkner M, Wang E, Merrill AH Jr, Rothman KJ, Hendricks KA. Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. *Environ Health Perspect* 2006;114:237–41.
9. Wang E, Norred WP, Bacon CW, Riley RT, Merrill AH Jr. Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with *Fusarium moniliforme*. *J Biol Chem* 1991;266:14486–90.
10. Stevens VL, Tang J. Fumonisin B1-induced sphingolipid depletion inhibits vitamin uptake via the glycosylphosphatidylinositol-anchored folate receptor. *J Biol Chem* 1997; 272:18020–5.
11. Flynn TJ, Stack ME, Troy AL, Chirtel SJ. Assessment of the embryotoxic potential of the total hydrolysis product of fumonisin B1 using cultured organogenesis-staged rat embryos. *Food Chem Toxicol* 1997;35:1135–41.
12. Sadler TW, Merrill AH, Stevens VL, Sullards MC, Wang E, Wang P. Prevention of fumonisin B1-induced neural tube defects by folic acid. *Teratology* 2002;66:169–76.
13. Gelineau-van Waes J, Starr L, Maddox J, Aleman F, Voss KA, Wilberding J, Riley RT. Maternal fumonisin exposure and risk for neural tube defects: mechanisms in an *in vivo* mouse model. *Birth Defects Res A Clin Mol Teratol* 2005;73:487–97.
14. Tamura T, Goldenberg R. Zinc nutriture and pregnancy outcome. *Nutr Res* 1996;16:139–81.
15. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev* 1993;73:79–118.
16. Bergmann KE, Makosch G, Tews KH. Abnormalities of hair zinc concentration in mothers of newborn infants with spina bifida. *Am J Clin Nutr* 1980;33:2145–50.
17. Srinivas M, Gupta DK, Rathi SS, Grover JK, Vats V, Sharma JD, Mitra DK. Association between lower hair zinc levels and neural tube defects. *Indian J Pediatr* 2001;68:519–22.
18. Carrillo-Ponce Mde L, Martínez-Ordaz VA, Velasco-Rodríguez VM, Hernández-García A, Hernández-Serrano MC, Sanmiguel F. Serum lead, cadmium, and zinc levels in newborns with neural tube defects from a polluted zone in Mexico. *Reprod Toxicol* 2004;19:149–54.
19. Golalipour MJ, Mansourian AR, Keshtkar A. Serum zinc levels in newborns with neural tube defects. *Indian Pediatr* 2006;43:809–12.
20. Hurley LS, Swenerton H. Congenital malformations resulting from zinc deficiency in rats. *Proc Soc Exp Biol Med* 1966;123: 692–6.
21. Sever LE. Zinc and human development: a review. *Hum Ecol* 1975;3:43–55.
22. Hambidge KM, Neldner KH, Walravens PA. Zinc, acrodermatitis enteropathica, and congenital malformations. *Lancet* 1975;305:577–8. [Letter]
23. Cavdar AO, Arcasoy A, Baycu T, Himmetoglu O. Zinc deficiency and anencephaly in Turkey. *Teratology* 1980;22:141.
24. Cavdar AO, Bahçeci M, Akar N, et al. Zinc status in pregnancy and the occurrence of anencephaly in Turkey. *J Trace Elem Electrolytes Health Dis* 1988;2:9–14.
25. Dinçer N, Akar N. Maternal hair zinc, copper and magnesium concentration in neural tube defects in Turkey. *Trace Elem Electrolytes* 1995;12:184–5.

26. Küry S, Dréno B, Bézieau S, Giraudet S, Kharfi M, Kamoun R, Moisan JP. Identification of *SLC39A4*, a gene involved in acrodermatitis enteropathica. *Nat Genet* 2002;31:239–40.
27. Küry S, Kharfi M, Kamoun R, et al. Mutation spectrum of human *SLC39A4* in a panel of patients with acrodermatitis enteropathica. *Hum Mutat* 2003;22:337–8.
28. Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. *Am J Hum Genet* 2002;71:66–73.
29. Dufner-Beattie J, Wang F, Kuo YM, Gitschier J, Eide D, Andrews GK. The acrodermatitis enteropathica gene *ZIP4* encodes a tissue-specific, zinc-regulated zinc transporter in mice. *J Biol Chem* 2003;278:33474–81.
30. Nakano A, Nakano H, Nomura K, Toyomaki Y, Hanada K. Novel *SLC39A4* mutations in acrodermatitis enteropathica. *J Invest Dermatol* 2003;120:963–6.
31. Wang F, Kim BE, Dufner-Beattie J, Petris MJ, Andrews G, Eide DJ. Acrodermatitis enteropathica mutations affect transport activity, localization and zinc-responsive trafficking of the mouse ZIP4 zinc transporter. *Hum Mol Genet* 2004;13: 563–71.
32. Maverakis E, Fung MA, Lynch PJ, Draznin M, Michael DJ, Ruben B, Fazel N. Acrodermatitis enteropathica and an overview of zinc metabolism. *J Am Acad Dermatol* 2007;56: 116–24.
33. McGrath JA, Bleck O. Acrodermatitis enteropathica. In: *NORD Guide to Rare Disorders. The National Organization for Rare Disorders*. Philadelphia: Lippincott Williams & Wilkins, 2003;94.
34. Miller SJ. Nutritional deficiency and the skin. *J Am Acad Dermatol* 1989;21:1–30.
35. Winton GB. Skin diseases aggravated by pregnancy. *J Am Acad Dermatol* 1989;20:1–13.
36. Pérez-Maldonado A, Kurban AK. Metabolic diseases and pregnancy. *Clin Dermatol* 2006;24:88–90.
37. Solomons NW, Jacob RA. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. *Am J Clin Nutr* 1981;34:475–82.
38. O'Brien KO, Zavaleta N, Caulfield LE, Wen J, Abrams SA. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. *J Nutr* 2000;130:2251–5.
39. Brenton DP, Jackson MJ, Young A. Two pregnancies in a patient with acrodermatitis enteropathica treated with zinc sulphate. *Lancet* 1981;318:500–2.
40. Akar N, Bahçeci M, Uçkan D, et al. Maternal plasma zinc levels after oral zinc tolerance test in pregnancies associated with neural tube defects in Turkey. *J Trace Elem Exp Med* 1991;4:225–7.
41. Cavdar AO, Bahçeci M, Akar N, Dinçer FN, Erten J. Maternal hair zinc concentration in neural tube defects in Turkey. *Biol Trace Elem Res* 1991;30:81–5.
42. Akar N. Pregnancy outcome of mothers with defective oral zinc tolerance test. *J Trace Elem Exp Med* 1997;10:153–4.
43. Velie EM, Block G, Shaw GM, Samuels SJ, Schaffer DM, Kulldorff M. Maternal supplemental and dietary zinc intake and the occurrence of neural tube defects in California. *Am J Epidemiol* 1999;150:605–16.
44. Groenen PM, Peer PG, Wevers RA, Swinkels DW, Franke B, Mariman EC, Steegers-Theunissen RP. Maternal myo-inositol, glucose, and zinc status is associated with the risk of offspring with spina bifida. *Am J Obstet Gynecol* 2003;189:1713–9.
45. Groenen PM, Klootwijk R, Schijvenaars MM, Straatman H, Mariman EC, Franke B, Steegers-Theunissen RP. Spina bifida and genetic factors related to myo-inositol, glucose, and zinc. *Mol Genet Metab* 2004;82:154–61.
46. Cengiz B, Söylemez F, Oztürk E, Cavdar AO. Serum zinc, selenium, copper, and lead levels in women with second-trimester induced abortion resulting from neural tube defects: a preliminary study. *Biol Trace Elem Res* 2004;97:225–35.
47. Bound JP, Harvey PW, Francis BJ, Awwad F, Gatrell AC. Involvement of deprivation and environmental lead in neural tube defects: a matched case-control study. *Arch Dis Child* 1997;76:107–12.
48. Macdonell JE, Campbell H, Stone DH. Lead levels in domestic water supplies and neural tube defects in Glasgow. *Arch Dis Child* 2000;82:50–3.
49. Brender JD, Suarez L, Felkner M, et al. Maternal exposure to arsenic, cadmium, lead, and mercury and neural tube defects in offspring. *Environ Res* 2006;101:132–9.
50. Balarajan R, McDowall M. Congenital malformations and agricultural workers. *Lancet* 1983;321:1112–3.
51. Blatter BM, Roeleveld N, Zielhuis GA, Gabreëls FJ, Verbeek AL. Maternal occupational exposure during pregnancy and the risk of spina bifida. *Occup Environ Med* 1996;53:80–6.
52. Blatter BM, Roeleveld N, Zielhuis GA, Mullaart RA, Gabreëls FJ. Spina bifida and parental occupation. *Epidemiology* 1996;7: 188–93.
53. Blatter BM, Roeleveld N. Spina bifida and parental occupation in a Swedish register-based study. *Scand J Work Environ Health* 1996;22:433–7.
54. Blatter BM, Hermens R, Bakker M, Roeleveld N, Verbeek AL, Zielhuis GA. Paternal occupational exposure around conception and spina bifida in offspring. *Am J Ind Med* 1997;32:283–91.
55. Blatter BM, Roeleveld N, Bermejo E, Martínez-Frías ML, Siffel C, Czeizel AE. Spina bifida and parental occupation: results from three malformation monitoring programs in Europe. *Eur J Epidemiol* 2000;16:343–51.
56. García AM, Fletcher T, Benavides FG, Orts E. Parental agricultural work and selected congenital malformations. *Am J Epidemiol* 1999;149:64–74.
57. Shaw GM, Velie EM, Katz EA, Morland KB, Schaffer DM, Nelson V. Maternal occupational and hobby chemical exposures as risk factors for neural tube defects. *Epidemiology* 1999;10:124–9.
58. Shaw GM, Nelson V, Olshan AF. Paternal occupational group and risk of offspring with neural tube defects. *Paediatr Perinat Epidemiol* 2002;16:328–33.
59. Nordby KC, Andersen A, Irgens LM, Kristensen P. Indicators of mancozeb exposure in relation to thyroid cancer and neural tube defects in farmers' families. *Scand J Work Environ Health* 2005;31:89–96.
60. Rull RP, Ritz B, Shaw GM. Neural tube defects and maternal residential proximity to agricultural pesticide applications. *Am J Epidemiol* 2006;163:743–53.
61. Lacasaña M, Vázquez-Grameix H, Borja-Aburto VH, Blanco-Muñoz J, Romieu I, Aguilar-Garduño C, García AM. Maternal and paternal occupational exposure to agricultural

work and the risk of anencephaly. *Occup Environ Med* 2006;63: 649–56.
62. Yang J, Carmichael SL, Canfield M, Song J, Shaw GM; National Birth Defects Prevention Study. Socioeconomic status in relation to selected birth defects in a large multicentered US case-control study. *Am J Epidemiol* 2008;167: 145–54.
63. Wasserman CR, Shaw GM, Selvin S, Gould JB, Syme SL. Socioeconomic status, neighborhood social conditions, and neural tube defects. *Am J Public Health* 1998;88:1674–80.
64. Farley TF, Hambidge SJ, Daley MF. Association of low maternal education with neural tube defects in Colorado, 1989–1998. *Public Health* 2002;116:89–94.
65. Meyer RE, Siega-Riz AM. Sociodemographic patterns in spina bifida birth prevalence trends–North Carolina, 1995–1999. *MMWR Recomm Rep* 2002;51:12–5.
66. Blanco Muñoz J, Lacasaña M, Borja Aburto VH, Torres Sánchez LE, García García AM, López Carrillo L. Socioeconomic factors and the risk of anencephaly in a Mexican population: a case-control study. *Public Health Rep* 2005;120: 39–45.
67. Jones KL. Fetal alcohol syndrome. In: Jones KL, ed. *Smith's Recognizable Patterns of Human Malformation*. Philadelphia: Elsevier Saunders, 2006;646–7.
68. Johnson VP, Swayze VW II, Sato Y, Andreasen NC. Fetal alcohol syndrome: craniofacial and central nervous system manifestations. *Am J Med Genet* 1996;61:329–39.
69. Clarren SK, Smith DW. Fetal alcohol syndrome. *N Engl J Med* 1978;298:1063–7.
70. Goldstein G, Arulanantham K. Neural tube defect and renal anomalies in a child with fetal alcohol syndrome. *J Pediatr* 1978;93:636–7.
71. Clarren SK. Neural tube defects and fetal alcohol syndrome. *J Pediatr* 1979;95:328.
72. Fuster JS, Guel S, Cahuana AB, Carcia-Tornel S. Neural tube defects and fetal alcohol syndrome. *J Pediatr* 1979;95:328–9.
73. Friedman JM. Can maternal alcohol ingestion cause neural tube defects? *J Pediatr* 1982;101:232–4.
74. Castro-Gago M, Novo I, Peña J. Maternal alcohol ingestion and neural tube defects: observation of four brothers in a family. *Brain Dev* 1987;9:321–2.
75. Kan DC, Tsai FJ, Peng CT, Tsai CH. Fetal alcohol syndrome with Arnold-Chiari malformation: report of one case. *Acta Paediatr Sin* 1998;39:116–8.
76. Mills JL, Graubard BI. Is moderate drinking during pregnancy associated with an increased risk for malformations? *Pediatrics* 1987;80:309–14.
77. McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and congenital defects. *Am J Public Health* 1992;82:91–3.
78. Shaw GM, Velie EM, Morland KB. Parental recreational drug use and risk for neural tube defects. *Am J Epidemiol* 1996;144:1155–60.
79. Chen SY, Sulik KK. Free radicals and ethanol-induced cytotoxicity in neural crest cells. *Alcohol Clin Exp Res* 1996;20: 1071–76.
80. Chen SY, Charness ME, Wilkemeyer MF, Sulik KK. Peptide-mediated protection from ethanol-induced neural tube defects. *Dev Neurosci* 2005;27:13–9.
81. Chen SY, Wilkemeyer MF, Sulik KK, Charness ME. Octanol antagonism of ethanol teratogenesis. *FASEB J* 2001;15: 1649–51.
82. Chen SY, Dehart DB, Sulik KK. Protection from ethanol-induced limb malformations by the superoxide dismutase/catalase mimetic, EUK-134. *FASEB J* 2004;18:1234–6.
83. Padmanabhan R, Ibrahim A, Bener A. Effect of maternal methionine pre-treatment on alcohol-induced exencephaly and axial skeletal dysmorphogenesis in mouse fetuses. *Drug Alcohol Depend* 2002;65:263–81.
84. Halsted CH, Villanueva J, Chandler CJ, et al. Ethanol feeding of micropigs alters methionine metabolism and increases hepatocellular apoptosis and proliferation. *Hepatology* 1996;23:497–505.
85. Polache A, Martin-Algarra RV, Guerri C. Effects of chronic alcohol consumption on enzyme activities and active methionine absorption in the small intestine of pregnant rats. *Alcohol Clin Exp Res* 1996;20:1237–42.
86. Muldoon RT, McMartin KE. Ethanol acutely impairs the renal conservation of 5-methyltetrahydrofolate in the isolated perfused rat kidney. *Alcohol Clin Exp Res* 1994;18:333–9.
87. McGuffin R, Goff P, Hillman RS. The effect of diet and alcohol on the development of folate deficiency in the rat. *Br J Haematol* 1975;31:185–92.
88. Tamura T, Halsted CH. Folate turnover in chronically alcoholic monkeys. *J Lab Clin Med* 1983;101:623–8.
89. Yanaguita MY, Gutierrez CM, Ribeiro CNM, Lima GA, Machado HR, Peres LC. Pregnancy outcome in ethanol-treated mice with folic acid supplementation in saccharose. *Childs Nerv Syst* 2008;24:99–104.
90. Ahlgren SC, Thakur V, Bronner-Fraser M. Sonic hedgehog rescues cranial neural crest from cell death induced by ethanol exposure. *Proc Natl Acad Sci U S A* 2002;99:10476–81.
91. Yamada Y, Nagase T, Nagase M, Koshima I. Gene expression changes of Sonic hedgehog signaling cascade in a mouse embryonic model of fetal alcohol syndrome. *J Craniofac Surg* 2005;16:1055–61.
92. Kibar Z, Vogan KJ, Groulx N, Justice MJ, Underhill DA, Gros P. Ltap, a mammalian homolog of *Drosophila* Strabismus/Van Gogh, is altered in the mouse neural tube mutant Loop-tail. *Nat Genet* 2001;28:251–5.
93. Katoh M. Strabismus (*STB*)/Vang-like (*VANGL*) gene family. *Int J Mol Med* 2002;10:11–5.
94. Kibar Z, Torban E, McDearmid JR, et al. Mutations in VANGL1 associated with neural-tube defects. *N Engl J Med* 2007;356:1432–7.
95. Torban E, Patenaude AM, Leclerc S, et al. Genetic interaction between members of the Vangl family causes neural tube defects in mice. *Proc Natl Acad Sci U S A* 2008;105:3449–54.
96. Amorosi S, D'Armiento M, Calcagno G, et al. *FOXN1* homozygous mutation associated with anencephaly and severe neural tube defect in human athymic Nude/SCID fetus. *Clin Genet* 2008;73:380–4.
97. Pignata C, Fiore M, Guzzetta V, Castaldo A, Sebastio G, Porta F, Guarino A. Congenital alopecia and nail dystrophy associated with severe functional T-cell immunodeficiency in two sibs. *Am J Med Genet* 1996;65:167–70.
98. Frank J, Pignata C, Panteleyev AA, et al. Exposing the human nude phenotype. *Nature* 1999;398:473–4.

99. Brissette JL, Li J, Kamimura J, Lee D, Dotto GP. The product of the mouse nude locus, *Whn*, regulates the balance between epithelial cell growth and differentiation. *Genes Dev* 1996;10:2212–21.
100. Suzuki N, Hirata M, Kondo S. Traveling stripes on the skin of a mutant mouse. *Proc Natl Acad Sci U S A* 2003;100:9680–5.
101. Danishpajooh IO, Gudi T, Chen Y, Kharitonov VG, Sharma VS, Boss GR. Nitric oxide inhibits methionine synthase activity in vivo and disrupts carbon flow through the folate pathway. *J Biol Chem* 2001;276:27296–303.
102. Traister A, Abashidze S, Gold V, Plachta N, Karchovsky E, Patel K, Weil M. Evidence that nitric oxide regulates cell-cycle progression in the developing chick neuroepithelium. *Dev Dyn* 2002;225:271–6.
103. Weil M, Abeles R, Nachmany A, Gold V, Michael E. Folic acid rescues nitric oxide-induced neural tube closure defects. *Cell Death Differ* 2004;11:361–3.
104. 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.
105. Dahiyat M, Cumming A, Harrington C, et al. Association between Alzheimer's disease and the *NOS3* gene. *Ann Neurol* 1999;46:664–7.
106. Akomolafe A, Lunetta KL, Erlich PM, et al. Genetic association between endothelial nitric oxide synthase and Alzheimer disease. *Clin Genet* 2006;70:49–56.
107. Kobashi G, Yamada H, Ohta K, Kato EH, Ebina Y, Fujimoto S. Endothelial nitric oxide synthase gene (*NOS3*) variant and hypertension in pregnancy. *Am J Med Genet* 2001;103:241–4.
108. Yoshimura T, Yoshimura M, Tabata A, Yasue H, Okamura H. The missense Glu298Asp variant of the endothelial nitric oxide synthase gene is strongly associated with placental abruption. *Hum Genet* 2001;108:181–3.
109. Hillermann R, Carelse K, Gebhardt GS. The Glu298Asp variant of the endothelial nitric oxide synthase gene is associated with an increased risk for abruptio placentae in pre-eclampsia. *J Hum Genet* 2005;50:415–9.
110. Jachymova M, Horky K, Bultas J, Kozich V, Jindra A, Peleska J, Martasek P. Association of the Glu298Asp polymorphism in the endothelial nitric oxide synthase gene with essential hypertension resistant to conventional therapy. *Biochem Biophys Res Commun* 2001;284:426–30.
111. Casas JP, Bautista LE, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase genotype and ischemic heart disease: meta-analysis of 26 studies involving 23028 subjects. *Circulation* 2004;109:1359–65.
112. Yoshimura M, Yasue H, Nakayama M, et al. A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. *Hum Genet* 1998;103:65–9.
113. Hingorani AD, Liang CF, Fatibene J, et al. A common variant of the endothelial nitric oxide synthase (Glu^{298}→Asp) is a major risk factor for coronary artery disease in the UK. *Circulation* 1999;100:1515–20.
114. Berger K, Stogbauer F, Stoll M, et al. The glu298asp polymorphism in the nitric oxide synthase 3 gene is associated with the risk of ischemic stroke in two large independent case-control studies. *Hum Genet* 2007;121: 169–78.
115. Brown KS, Cook M, Hoess K, Whitehead AS, Mitchell LE. Evidence that the risk of spina bifida is influenced by genetic variation at the *NOS3* locus. *Birth Defects Res A Clin Mol Teratol* 2004;70:101–6.
116. van der Linden IJM, Heil SG, den Heijer M, Blom HJ. The 894G>T variant in the endothelial nitric oxide synthase gene and spina bifida risk. *J Hum Genet* 2007;52: 516–20.
117. Brown KS, Kluijtmans LA, Young IS, et al. Genetic evidence that nitric oxide modulates homocysteine: the *NOS3* 894TT genotype is a risk factor for hyperhomocysteinemia. *Arterioscler Thromb Vasc Biol* 2003;23:1014–20.