Spina Bifida and Other Neural Tube Defects

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Neural tube defects (NTDs) are the most common severely disabling birth defects in the United States, with a frequency of approximately 1 of every 2000 births. NTDs include all congenital anomalies that involve failure of the neural tube to close during the fourth week of embryogenesis. NTDs can occur anywhere along the formation of the spinal cord, from the brain to the sacrum. The term neural tube defects is most often used to refer to congenital defects of the central nervous system, which involve exposed nervous tissue such as craniorachischisis (exposure of the entire central nervous system), anencephaly (exposed or absent brain), meningomyelocele (an exposed area of spinal cord), or encephalocele (a protrusion of meningeal or skin covered brain). The majority of NTDs result in either anencephaly or meningomyelocele, with each defect seen in almost equal proportions at birth. Anencephaly is a lethal defect, characterized by acrania and rudimentary or absent cerebral hemispheres and cerebellum. Meningomyelocele, also called spina bifida or spina bifida cystica, is compatible with life but results in handicap approximately 99% of the time. In meningomyelocele, protrusion of the spinal cord and meninges through a defect in the vertebral arch can occur anywhere along the spinal column but is most common in the lumbar region. Skin covers 15% to 20% of NTDs; when the sac contains meninges and cerebrospinal fluid, but the spinal cord and meninges remain entirely within the vertebral canal, the defect is referred to as a meningocele. Spina bifida cystica is more common than meningocele and results in a marked neurologic deficit inferior to the level of the protruding sac.

Although spina bifida occurs most commonly in the lumbar regions of the spine, it can occur at any level. Spina bifida ranges from minor types (spina bifida occulta) to severe, clinically significant types (spina bifida cystica). People with spina bifida cystica have medical problems and physical handicaps that dramatically impact their lives and the lives of their families. Depending on the level of the lesion, interruption of the spinal cord at the site of the defect causes paralysis of the legs, incontinence of urine and feces, anesthesia of the skin, and abnormalities of the hips, knees, and feet.

Spina Bifida

Spina Bifida Occulta

Spina bifida occulta ("hidden") is the mildest form of spina bifida, resulting from a gap in one or more vertebral arches, but the spinal cord and meninges remain entirely within the vertebral canal. Spina bifida occulta may manifest as a small cavity (dermal sinus) between 2 adjacent vertebrae, indicating that the vertebrae did not fuse properly; evidence of this defect may be a small dimple with a hairy patch or birthmark above it. When this abnormality involves failure of only a single vertebra to fuse and the spinal cord and spinal nerves are normal, neurologic symptoms are commonly absent, producing no clinical symptoms. In fact, spina bifida occulta occurs in lower lumbar or sacral vertebrae in approximately 10% of otherwise healthy people, thus being regarded as a normal variation in the population. However, if several vertebrae are involved in this milder type of NTD, bowel, bladder, or motor problems may eventually develop.

Spina Bifida Cystica

Severe types of spina bifida involve protrusion of the spinal cord and/or meninges through a defect in the vertebral arch and are referred to as spina bifida cystica because of the cyst-like sac that is associated with these
malformations. When the sac contains meninges and cerebrospinal fluid, but the spinal cord and spinal root are in their normal position, the defect is referred to as a meningocele. Despite their normal location, spinal cord abnormalities can be seen with the meningocele. In meningomyelocele, the spinal cord and/or nerve roots protrude through the defect in the vertebral arch and are included in the sac. Meningomyelocele is a more common and more severe malformation than meningocele.

Depending on the level of the lesion, interruption of the spinal cord at the site of the meningomyelocele defect causes paralysis of the legs, incontinence of urine and feces, anesthesia of the skin, and abnormalities of the hips, knees, and feet. In fact, only 1% of children born with an open NTD are free of handicap. Children born with a meningomyelocele need multiple surgeries and invasive procedures. For example, surgery is needed within 24 to 48 hours after birth to repair an open defect in order to reduce the risk of infection. Without this surgery, only 20% of these infants survive to age 2 years. Most affected individuals have an associated deformity at the base of the brain, the Arnold-Chiari type II malformation, which probably accounts for the well-established hydrocephalus present at birth in about 80% of cases. Although mental deficiency occurs infrequently in children with meningomyelocele, the brain and spinal cord abnormalities associated with spina bifida also affect learning. Most children have normal intelligence but show problems with perceptual motor skills, attention, memory, and organization.

Neural Tube Development

Neurulation

Neural tube formation and closure involve complex cellular, extracellular, and intercellular processes. Formation begins with primary neurulation and is completed by the process of canalization, which occurs during secondary neurulation. Primary neurulation begins when the notochord induces the overlying embryonic ectoderm to form a cellular plate. The notochord defines the primitive axis of the embryo, whereas the cellular neural plate will form the neural tube and is the precursor of the central nervous system (brain and spinal cord). As the notochord forms and elongates, the neural plate broadens and extends beyond the edges of the notochord. On approximately day 18 in human fetal development, the neural plate begins to invaginate along its central axis, forming a longitudinal median groove with neural folds on each of its sides. The midline of the neural plate becomes anchored to the underlying axial mesoderm, establishing a hinge around which the lateral folds elevate. One of the first steps in elevation of the neural folds is the development of hinge points, which are formed through alterations in neuroepithelial cell shape called wedging. The forces of cell wedging, migration, and expansion of the surface ectoderm, as well as growth and expansion of the underlying mesoderm, are all factors that provide the mechanical impetus to bring the lateral halves of the neural plate to the midline. By days 22 to 24 of development, the process of elevation is followed by fusion of the neural folds, converting the neural plate into the neural tube. Upon initial closure, the neural tube has openings at either end—the anterior and posterior neuropores—which allow for the fluid of the amniotic cavity to communicate with the lumen of the neural tube. The anterior neuropore closes on days 24 to 26, followed by posterior neuropore closure on days 25 to 28 of development.

Primary neurulation results in a closed neural tube with its caudal limit in the upper lumbar spinal cord. The caudal portion of the neural tube does not arise by fusion of the neural folds but develops from a cellular mass known as the caudal eminence. Once the posterior neuropore is closed, neural tissue is laid down as a neural cord into which the cavity of the more rostral, newly developed neural tube extends. The process by which the remaining most caudal elements of the spinal cord are formed is known as canalization, or secondary neurulation, and corresponds to the future vertebral level 8.

Closure Theories

Two primary theories exist regarding the fusion sites and the timing of neural tube formation. First, the traditional “zipper model” states that the neural tube closes in a continuous, bidirectional process. According to this model, the neural folds first meet and close in the cervical region, and fusion then proceeds bidirectionally until a tube is formed.

In 1993, Van Allen et al. proposed the second theory of neural tube closure, stating that the human neural tube closes at multiple locations. The “multi-site closure model” states that the process of neural fold fusion is initiated at 5 sites along the future neural tube. Van Allen et al. propose that multi-site neural tube closure provides the best explanation for NTDs in humans, and
several recent studies have provided additional evidence supporting this model of neural tube closure. According to the multi-site closure model, the initial site of closure (closure 1) is in the midcervical region and proceeds cranially and caudally, closing over the area of the future spine at the level of L2. Closure 2 begins at the prosencephalon/mesencephalon boundary and proceeds bidirectionally, whereas closure 3 proceeds rostrally from the stomodeum and meets the cranial end of closure site 2. Closure 4 takes place in the region of the rhombencephalon and proceeds rostrally. Finally, closure 5 unidirectionally closes the caudal end of the neural tube from the level of future S2 through L2. Failure of the neural tube to close within these 5 sites can explain all types of NTDs, with spina bifida resulting from incomplete fusion of closure 5 or of rostral or caudal closure 1.

Abnormal Neurulation

As stated earlier, in normal neural tube development the edges of the neural plate fold toward each other and fuse to form the neural tube. As the neural plate develops into the spinal cord, bone and muscle form a protective barrier around it. Spina bifida results from abnormal neurulation in which a portion of the neural plate fails to join together, and therefore bone and muscle are unable to grow over this open section of the developing spinal column. The result is a “hole” in the back through which the spinal cord and/or meninges (nerve tissues) protrude. The severity of symptoms is determined by the particular nerves involved (the level of the defect) and their degree of damage and/or maldevelopment. Children with meningomyelocele have neurologic deficits at the level of the defect and below, resulting in varying degrees of muscle paralysis, bladder and bowel problems, loss of skin sensation, and spine and limb deformities.

Management and Care

Prenatal Detection

Alpha fetoprotein (AFP) is a protein present in fetal tissues during development and is utilized for prenatal diagnosis of NTDs. As the embryo develops, closure of the abdominal wall and neural tube prevents release of AFP into the amniotic fluid. If an NTD is present, AFP produced by the fetus leaks into the amniotic fluid through the defect and diffuses into maternal circulation and can be detected in maternal serum, thus allowing for prenatal diagnosis of NTDs. Measurement of maternal AFP is a standard part of the “triple screen” tests performed on pregnant women during their first trimester. The triple screen test can identify approximately 75% to 80% of meningomyelocele-complicated pregnancies at 16 weeks’ gestation. If an elevated maternal AFP level is detected, amniocentesis is performed to check the amniotic fluid for AFP; ultrasonography of the fetus is then used to confirm the diagnosis and evaluate the fetus for anomalies.

Delivery and Neurosurgery

Although somewhat controversial, the majority of studies support the delivery of babies with spina bifida by planned cesarean section, thus allowing more careful delivery of the baby to protect the spinal cord from injury and to prevent possible rupture of the meningeal sac. Once a child with a meningomyelocele is delivered, it is necessary to repair the defect within 24 to 48 hours to reduce the risk of infection. Initial evaluations of the newborn are important to assess the potential complications (including the presence of hydrocephalus, deformities of the legs, and leg-movement problems) and include a sensory examination to determine the level of the lesion and prognosis for motor ability and a urologic evaluation to determine continence.

Children born with a meningomyelocele have abnormalities that are not limited to the spinal cord, including the development of hydrocephalus and the development of symptoms related to the area where the brain and spinal cord join (Arnold-Chiari malformation). Approximately 85% to 90% of babies with spina bifida either have hydrocephalus at birth or develop it soon after. Hydrocephalus is a condition in which an enlargement of the ventricular system of the brain occurs because of an imbalance between the production and absorption of cerebrospinal fluid. The higher the level of lesion, the greater the incidence of hydrocephalus. Hydrocephalus is alleviated by utilization of a shunt to provide drainage to the blocked ventricles. A shunt is a small flexible tube inserted through a small opening in the skull, leading from the brain ventricle underneath the skin of the head and neck, to drain excess cerebrospinal fluid from the brain into the abdomen where it is absorbed. Two common problems with shunts are malfunction and infection, both of which can be treated by shunt revision. There are conflicting views concerning whether a child with hydro-
Cephalus should have a shunt procedure performed. Studies of intellectual functioning of children with meningomyelocele have shown that children who have never had a shunt procedure generally do better intellectually than children who have had a shunt procedure. However, interpretation of these studies is difficult because the children with shunts had more severe hydrocephalus initially, which could be the determinant of their limited intellectual functioning. An additional study concluded that it is the revision of shunts, particularly after age 2 years, that is associated with poor long-term achievement in adults with spina bifida.

The Arnold-Chiari type II malformation affects the hindbrain and upper cervical spine and is characterized by a downward herniation of the cerebellum and portion of the brain stem into the cervical spine. The vast majority of patients with spina bifida exhibit some degree of this malformation, with approximately one third of children who are born with a meningomyelocele developing symptomatic Arnold-Chiari malformation; although the symptoms resolve for most patients, a third of those who remain symptomatic will die (approximately 12% of the total). The development of symptoms related to the Arnold-Chiari malformation appears to be related to the severity of the malformation, which is primarily determined by the degree of descent of the brain into the cervical spine. Symptoms typically come from 1 of 3 areas of the central nervous system, including the cerebellum, the lower brain stem, and/or the spinal cord. Lower brain-stem symptoms most often present in newborns and young infants include difficulty swallowing, inspiratory stridor, weak or poor cry, and sustained arching of the head. When severe, these symptoms may result in insufficient breathing to maintain life, making symptomatic Arnold-Chiari malformation the leading cause of death in children with spina bifida. As a child with Arnold-Chiari malformation progresses into adolescence, he or she may develop stiffness or spasticity of the arms and hands along with a loss of feeling or sensation, often due to an abnormality of the spinal cord. Symptoms arising from the cerebellum are least likely to occur and produce problems with balance and coordination.

Controversy exists regarding proper management of the Arnold-Chiari malformation; however, it is generally accepted that the initial step be directed toward relieving intracranial pressure by use of a shunt. Most patients with Arnold-Chiari malformation do not need surgery unless serious symptoms are present, in which case surgical intervention should be considered. The surgical approach consists of posterior fossa decompression and laminectomy and the establishment and maintenance of normal movement of the cerebrospinal fluid. Griebel et al. state that "because of the reversibility of potentially lethal symptoms in some patients [with Arnold-Chiari] and because of the low risks involved in surgery, we feel that posterior fossa decompression and laminectomy is justified in children with serious brain stem symptoms."

**Orthopedics**

The question of whether a child with spina bifida will be able to walk is most dependent upon the lesion level of the meningomyelocele. Children with low-level lesions (low lumbar and sacral levels) are usually able to walk, although they may need the help of braces and/or crutches. Patients with midlevel lesions (midlumbar level) typically require significant support in the form of braces, twister cables, crutches, or walkers to walk for even brief periods. Most patients with lesions at the upper lumbar level and above require wheelchairs for mobility. The percentage of children who can walk within each lesion-level group is estimated to be: sacral, 100%; low lumbar, 95%; high lumbar, 30%; and thoracic, 33%. The types of orthopedic problems a person with spina bifida is faced with include clubfoot, hip dysplasia, and spinal deformities such as scoliosis and kyphosis. Developmental or paralytic spinal deformities such as scoliosis (lateral bend of the spine) and kyphosis (forward bending of the lower spine) require orthotic support in younger patients in the form of bracing, with a possibility of surgical treatment during the preteen years. The primary cause of hip abnormalities in meningomyelocele patients is muscle imbalance, which produces abnormal forces across the hip joint. Prolonged sitting, because of lack of mobility, can lead to flexion deformities of the knee observed in 50% of patients with upper level lesions, 20% of lumbar lesions, and 15% of sacral lesions. The frequency of various foot deformities, including clubfoot (misalignment of the bones in the front part of the foot resulting in an abnormal shape), that occurs with meningomyelocele is quite high and primarily caused by muscle imbalance.

**Neuropsychology**

The brain and spinal cord abnormalities associated with spina bifida also affect learning, typically dis-
played through problems with perceptual-motor skills, numerical reasoning, attention, memory, and organization. Although most children with spina bifida have normal intelligence, they do have particular learning disabilities or weaknesses that often make it difficult for them to obtain substantive education, gainful employment, and autonomy in daily living. These weaknesses include the following: poor eye-hand coordination (perceptual-motor), may speak well but not be able to explain well or fully understand (comprehension), easily distracted (attention), restlessness (hyperactivity), trouble remembering what is said or seen (memory), disorganized (organization), unable to keep things in order (sequencing), and difficulty in making decisions and solving problems (reasoning).

The role of educational environments in assisting children with spina bifida to develop skills necessary for autonomy in adulthood is especially important. Evidence suggests that children with spina bifida who are enrolled in regular, integrated school environments composed of both physically handicapped and non-handicapped students function better academically, psychologically, and socially than their counterparts in specialized schools. If children and adolescents with spina bifida participate in individualized educational programs designed to reinforce academic strengths, increase social confidence and independent living skills, and provide options for future work, they will be more prepared to cope with and handle the many challenges of adulthood.

Spina Bifida as a Multifactorial Disease

Genetic Factors

Genetic or inherited factors are important to uncover the etiology of NTDs (Table 1). The case for the existence of a genetic predisposition for NTDs is supported by several lines of evidence including that (1) the incidence of NTDs varies greatly among populations and ethnic groups, (2) the risk of occurrence of NTDs in offspring of parents with prior affected pregnancies is substantially higher not only for those parents but also for their first- and second-degree relatives, and (3) alterations in genes involved in folate metabolism are associated with increased risk for NTDs.

The incidence of NTDs varies worldwide, from about 1 to about 9 per 1000 total births in different ethnic groups and in different parts of the world. For example, some of the groups with the highest incidence of NTDs are populations from southern Wales (7.6 per 1000) and Northern Ireland (8.6 per 1000). The general population risk for NTDs in the United States is approximately 1 per 2000, with spina bifida representing roughly half of these defects. Interestingly, the incidence of NTDs varies with geographical location within the United States. Greenberg et al found that the risk for spina bifida generally decreases from east to west, with the highest rate of spina bifida seen in southern Appalachia (0.8 per 1000) and the lowest rate seen in the Rocky Mountain states and the Pacific Northwest (0.1 per 1000).

The incidence of NTDs varies with ethnicity and geographical location within the United States. African Americans have a lower rate of NTDs than whites, whereas Asian Americans have been shown to have a lower incidence than either African Americans or whites. Hispanic persons in the United States, particularly in Texas, have a higher risk for having a child with an NTD than other ethnic groups in the United States.

A genetic contribution to malformations of the neural tube is suggested by familial recurrence risks and an altered sex ratio. The heritability for spina bifida is estimated to be 60%. Heritability is defined as the proportion of phenotypic variation in a population due to genetic variation. In other words, heritability is the proportion of a person’s outward, visible expression of their genes that is due solely to their genetic makeup and excludes environmental or other factors.

NTDs cluster in families. The recurrence risk is reflected by the local population prevalence and influenced by the number of family members affected, with the recurrence risk rising as the number of affected persons in a family increases. In the general population of the United States, the occurrence of NTDs in siblings is approximately 3%, and it roughly doubles with the birth of each additional child with an NTD. In second-degree relatives, the recurrence drops to 0.5%, and in third-degree relatives, it is approximately equal to the general population’s background risk (~1 per 2000).

An excess of females has been seen among children with NTDs. However, an equal sex ratio of children affected with spina bifida was observed in a study of 2 predominantly Hispanic counties bordering Mexico and in an additional study published on Hispanics in Houston, Texas. If a female predominance of this type of birth defect exists, it could result from in utero...
TABLE 1. Genetic and environmental factors associated with neural tube defects

<table>
<thead>
<tr>
<th>Genetic factors</th>
<th>Environmental factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTD incidence varies among ethnic groups</td>
<td>Maternal and paternal age*</td>
</tr>
<tr>
<td>NTD incidence with geographical location</td>
<td>Low socioeconomic class*</td>
</tr>
<tr>
<td>Familial recurrence risks</td>
<td>Parental occupation*</td>
</tr>
<tr>
<td>Altered sex ratio*</td>
<td>Maternal obesity and diabetes</td>
</tr>
<tr>
<td>NTD-susceptible animal models (see Table 3)</td>
<td>Maternal hyperthermia and illness</td>
</tr>
<tr>
<td>Genetic syndromes associated with NTDs</td>
<td>Maternal anticonvulsant drug use</td>
</tr>
</tbody>
</table>

*Conflicting results from multiple studies as to effect of these variables.

selection against affected males, leading to an increase in miscarriage rates and a decrease in the male-to-female ratio of siblings. In contrast, if a male protective mechanism against NTDs exists, the presence of a male proband suggests higher genetic susceptibility in a family. Similarly, families with multiple affected members are thought to have greater susceptibility to these NTDs. Therefore, families with male probands or multiple affected members have been investigated to determine whether these families have a higher fetal mortality rate, presumably of affected children. The rate of miscarriage has not been found to be significantly higher in families with a male proband or multiple affected members.

Several animal models that are susceptible to NTD malformations further suggest an etiologic role for genetics in the occurrence of this birth defect. First, homozygous mice with mutations in the Loop-tail (Lp) gene have craniorachischisis, in which the neural tube remains open from the midbrain to the tail. Secondly, curly-tail mutant mice are more likely to develop exencephaly with or without lumbosacral spina bifida. Next, the mouse mutant splotch, which develops exencephaly, meningocele, and spina bifida, has a mutation in the Pax3 gene. Finally, a study by Helwig et al. showed that digenic inheritance is one possible mechanism to explain the inheritance of NTDs. By crossing the Patch and undulated mutant mice, it was demonstrated that mice, who were homozygous for the undulated mutation and hemizygous for the Patch mutation, developed spina bifida. Neither of these mutant mouse strains independently have an inherited susceptibility for developing this type of NTD.

Specific chromosome abnormalities and genetic syndromes are associated with NTDs, further suggesting an etiologic role for genetics. Spina bifida occurs more often than expected in both trisomy 13 and trisomy 18. Additionally, NTDs are associated with many genetic syndromes, including acrocallosal syndrome, cerebrocostomandibular syndrome, CHILD syndrome, Fraser syndrome, Jarcho-Levin syndrome, Meckel-Gruber syndrome, and Waardenburg syndrome types I and II.

Environmental Factors

In addition to genetic factors, environmental influences are also important for understanding the etiology of NTDs (Table 1). In the epidemiologic study of NTDs, a broad concept of the environment has been utilized to embrace all nongenetic aspects of etiology, including maternal age, social class, and metabolic disease. Parental age, occupation, education, and socioeconomic status have been explored as potential risk factors. Additionally, pregnancy histories, maternal health, and maternal nutritional status have been investigated to examine their potential roles.

The role of maternal and paternal age in the etiology of NTDs is poorly understood. Multiple studies have examined maternal and/or paternal age in NTDs. Several studies have shown an increased risk with an increased maternal age. Strassburg et al. noted a general pattern of increasing risk with increasing maternal age for spina bifida in a study in Los Angeles County. In contrast, other studies found no relationship between NTDs and maternal age.

Low socioeconomic class, as measured by parental occupation and education, has been found to be associated with an increased risk for having an NTD in some studies. Low maternal educational level was found to be associated with anencephaly, but not spina bifida, in a study on risk factors in the Hispanic population of Harris County, Texas. A case-control study in Los Angeles revealed no association between socioeconomic class, as measured by income and parental education, and risk for NTD-affected offspring. Additionally, no association was found between risk and socioeconomic class in an
investigation in Cameron County, Texas. The mixed results of the available studies make it difficult to draw conclusions about a relationship between socioeconomic status and risk for NTDs.

The influence of parental occupation on the risk for offspring with congenital anomalies of the central nervous system has been investigated, but no definitive conclusions have been reached. An increased risk among children of parents with occupations involving exposure to solvents, such as industrial workers or painters, has been reported. Health care workers, primarily female nurses, have been shown to have an increased risk for offspring with NTDs. Agricultural occupations have also been shown to be associated with an increased risk for this type of birth defect. Finally, a significantly increased risk for NTDs has been seen among offspring of both mothers and fathers working in transportation-related occupations.

Two lines of evidence support the importance of glucose metabolism in the formation of NTDs: obese women are more likely to have offspring with NTDs (especially spina bifida), and women with diabetes are more likely to have offspring with NTDs. Several recent reports have provided evidence that maternal obesity is a risk factor for congenital malformations involving the central nervous system. The association appears to be independent of folate intake (see following) and is not readily explained by any known social, dietary, or medical confounders, therefore suggesting the increased risk might arise from a specific pathophysiologic disturbance of obesity. A study by Waller et al demonstrated that, when compared to women of normal weight, women who were obese before pregnancy (body mass index > 29 kg/m) showed a significantly increased risk of having an infant with an NTD (odds ratio [OR] = 1.8). The effect was particularly strong for spina bifida (OR = 2.6). A recent study by Shaw et al confirmed these findings. The study by Shaw et al also examined potentially confounding factors such as maternal non-use of vitamins containing folic acid, use of diet pills, lower dietary folate intake, diabetes, and an NTD-pregnancy history. It was determined that none of these factors accounted for the findings.

Maternal diabetes is known to be associated with an increased risk for offspring with NTDs. The risk has been estimated to range from as low as 2% in the United States to as high as 8% in Birmingham, England. Increased risk for both anencephaly and spina bifida has been noted. Anencephaly occurs in 2% to 5% of offspring of mothers with pregestational diabetes (both Type I and Type II). Additionally, the risk is increased among both white and African American populations in the United States. The recurrence risk for mothers with diabetes in the United States is 4%, similar to the risk for mothers without diabetes. The etiology underlying the increased risk has yet to be elucidated. Metabolic derangements, particularly altered glucose metabolism during organogenesis, are thought to be the mechanism; for example, aberrant glycosylation in rats results in birth defects similar to those seen in children born to mothers with diabetes.

Nutritional factors have been associated with NTD formation. Research data suggest that maternal vitamin deficiencies, especially of folate, play a role in the pathogenesis of NTDs. In fact, several studies have demonstrated that folic acid supplementation both before and during early pregnancy may prevent up to 70% of all NTDs. Folate acts both as a cofactor for enzymes involved in DNA and RNA synthesis and in the supply of methyl groups to the methylation cycle. Thus, a folate deficiency can lead to defective cell proliferation and cell death due to the inhibition of DNA synthesis and can cause a shortage of methionine, which will prevent cells from methylating proteins, lipids, and myelin. In fact, minor deficiencies of any essential nutrient may result in malformations of the developing fetus. Despite the obvious relationship between maternal folate status and occurrence of NTDs, it is unclear how folate is involved in the pathogenesis of NTDs.

Zinc is an additional nutrient essential for normal fetal growth and development because it facilitates gene transcription and is necessary for cell division, development, and differentiation. Inadequate zinc intake has been associated with NTDs in both animals and humans. In laboratory animals, severely reduced zinc intake for 1 to 2 days early in gestation was associated with the development of NTDs. In humans, women with an extremely rare genetic disorder of zinc metabolism—acrodermititis enteropathica—are at high risk for bearing children with NTDs. In a recent study by Velic et al increased total preconceptional zinc intake, as well as increased servings of animal products (the most bioavailable food source of zinc), was associated with a reduced risk for NTDs. Their analyses indicate that risk of NTDs in fetuses decreases with increased maternal preconceptional zinc intake; however, it remains unclear whether increased
TABLE 2. Allele frequencies of the MTHFR C677T mutation among NTD patients and controls in different ethnic populations

<table>
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<th>Population</th>
<th>Examined persons</th>
<th>677T allele frequency (T)</th>
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<tr>
<td></td>
<td>Mothers</td>
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<td>Hispanic(^{96})</td>
<td>Level 1 SB patients</td>
<td>0.527</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Mothers</td>
<td>0.562</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.473</td>
<td></td>
</tr>
</tbody>
</table>

SB, Spina bifida.

zinc intake or another nutrient or combination of nutrients correlated with dietary zinc intake is causally associated with reduced NTD risk.\(^{67}\)

Maternal illnesses and hyperthermia have been associated with an increased risk of NTDs. In 1992, Milunsky et al\(^{68}\) investigated the effect of maternal hyperthermia on NTD risk and found that exposure to a sauna, fever, electric blanket, or hot tub in early pregnancy increased the risk of NTD-affected offspring. In a retrospective, interview, case-control study, Sandford et al\(^{69}\) found that a significantly higher number of women reported taking hot baths during the critical period of neural tube formation. Finally, a report from the metropolitan Atlanta area found an increased risk for NTD to be associated with maternal reports of having “flu” with fever over periods ranging from 1 month before conception to 2 months after conception.\(^{70}\)

Anticonvulsant use has been found to be associated with an increased risk for malformed offspring.\(^{71}\) The malformations documented in the children of mothers with epilepsy do not generally include NTDs; however, maternal valproic acid use during early pregnancy has been demonstrated to increase a woman’s risk of having a child with spina bifida.\(^{71-73}\) Valproic acid, or sodium valproate, was the only anticonvulsant associated with an increased risk for offspring with spina bifida reported in one international study.\(^{26}\) Additionally, the association between valproic acid and spina bifida has been confirmed in separate studies in Spain, France, Italy, and Western Australia.\(^{74-77}\) The majority of these reports are of spina bifida involving the lower lumbar or sacral regions of the spine.

**Research for Disease-Causing Genes**

**Folate Metabolism**

The most recent development in uncovering the molecular basis of NTDs is the discovery of a genetic link to the most well-known environmental cause of NTD formation, folate deficiency in pregnant women. It was definitively proven almost 10 years ago that periconceptional folic acid supplementation decreases the recurrence of NTDs, as well as the first occurrence of NTDs.\(^{78,79}\) The study by the Medical Research Council Vitamin Study Research Group\(^{78}\) assigned 1817 women at high risk of having a pregnancy with
TABLE 3. Mouse models of neural tube defects, their syntenic chromosomal location in humans, and the genes involved (when known)

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Mouse model phenotype</th>
<th>Human chromosome location</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuolated lens</td>
<td>Opaque white lenses; white belly spot;</td>
<td>1q21-q22</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>caudal spina bifida</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Curly tail</td>
<td>Exencephaly; myeloschisis; kinked tail</td>
<td>1p36.3</td>
<td>—</td>
</tr>
<tr>
<td>Splotch</td>
<td>Myelomeningiocoele; exencephaly</td>
<td>2q36</td>
<td>PAX3</td>
</tr>
<tr>
<td>Undulated</td>
<td>Vertebral column abnormalities</td>
<td>20p11.2</td>
<td>PAX1</td>
</tr>
<tr>
<td>Patch</td>
<td>White fur patches; mesodermal deficiency</td>
<td>4q11-q12</td>
<td>PDGFRA</td>
</tr>
<tr>
<td>Rachiterata</td>
<td>Small body size; short kinked tail</td>
<td>2q23-q31</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>thoraco-lumbar malformations; abnormal axis</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Truncate</td>
<td>Short/absent tail; missing sacral/</td>
<td>2p13-p12</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>lumbar vertebrae; degeneration of notochord</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>T(Brachyury)</td>
<td>Posterior mesodermal defects;</td>
<td>6q27</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>short/absent tail; gut and neural tube fusions</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Loop-Tail</td>
<td>Craniorachischisis</td>
<td>1q21-q23</td>
<td>—</td>
</tr>
<tr>
<td>Danforth's Short Tail</td>
<td>Short kinked tail; discontinuous notochord</td>
<td>10p</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>kidney defects</td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>

an NTD (due to their having a previous NTD-affected pregnancy) to 1 of 4 groups: folic acid, other vitamins, both folic acid and other vitamins, or neither folic acid nor other vitamins. After 1195 of these high-risk women had a completed pregnancy with a known outcome, it was found that 27 had an NTD-affected pregnancy; 6 occurring in the folic acid groups and 21 in the 2 groups not including folic acid. Therefore, folic acid gave a 72% protective effect. A subsequent study by Czeizel and Dudas designed to determine whether folic acid supplementation could reduce the risk of first occurrence of NTDs. A randomized, controlled trial of periconceptional multivitamin supplementation was established and the results included 2104 women who received vitamin supplementation and 2052 women who received trace element supplementation. Those women who received trace element supplementation had 6 cases of NTDs as opposed to no cases of NTDs in the vitamin supplementation group, with the difference being statistically significant. The efficacy of folate in prevention of NTDs is now so widely accepted the decision has been made by the FDA to supplement foods in the United States with folate.

The exact mechanism for the action of folate in prevention of NTDs has been a predominant focus; however, it is still unclear how folate is involved in the pathogenesis of NTDs. It is known that elevated levels of the amino acid homocysteine in pregnant women increases risk for NTDs, and because the metabolism of homocysteine and folate are interdependent, it has been postulated that these risk factors are connected. In 1995, van der Put et al reported a thermolabile mutation in the enzyme 5,10-methylentetrahydrofolate reductase (MTHFR), which results in a 50% reduction in enzyme activity. MTHFR is important because it converts 5,10-methylentetrahydrofolate to 5-methyltetrahydrofolate, which, along with vitamin B12, acts as a cofactor with methionine synthase in the conversion of homocysteine to methionine. The mutation in MTHFR (C677T, substitution of valine for alanine at nucleotide position 677) is associated with decreased enzyme activity, low plasma folate, and high plasma homocysteine. Van der Put et al studied the frequency of the C677T MTHFR mutation in a Dutch population of spina bifida-affected individuals, as well as parents of spina bifida-affected individuals, and compared them to a control group. Only 5% of control patients were homozygous for the mutation, whereas 16% of mothers, 10% of fathers, and 13% patients were homozygous for the MTHFR mutation. A follow-up study tested genotypes of 41 fibroblast cultures from NTD-affected fetuses and compared them with genotypes of 109 individuals in the general population; homozygosity for the mutation was associated with a 7.2-fold increased risk of NTDs.

Over the last several years, data from several studies on different ethnic groups have resulted in different conclusions about the role of the C677T mutation of the MTHFR gene as a risk factor for NTDs (Table 2). For example, the C677T MTHFR variant has been shown to be a risk factor for NTDs in Irish and Dutch populations, but no corresponding association has been detected in British or German populations. Epidemiologic studies of NTDs in the United States have demonstrated that Hispanics experience a prevalence of spina bifida that is 2.5 times or greater than...
that of non-Hispanics. An additional study on Hispanics of Mexican American descent evaluated maternal MTHFR genotypic risk for having a spina bifida child with either an upper-level or lower-level meningocele and found statistically significant evidence that the maternal C677T MTHFR homozygous mutant genotype is a risk factor for upper-level spina bifida defects in Hispanics.

The study of folate and its association with NTDs is an ongoing endeavor that has led to numerous studies of different constituents involved in the folate metabolism pathway. Additional studies have investigated mutations in various other genes involved in folate metabolism, including cystathionine β-synthase and methionine synthase, but with no significant results proving them to be risk factors for NTDs. Recent mouse model studies have determined that folate acid—binding protein Folbp1 has a critical role in folate homeostasis during development and that functional defects in the human homologue (FOLR1) of Folbp1 may contribute to similar defects in humans. Even after dozens of studies, many basic questions on the role of folate and MTHFR in health and disease remain unanswered; thus the search for other genes, such as those coding for folate receptors or enzymes (including methylenetetrahydrofolate dehydrogenase [MTHFD] or serine hydroxymethyltransferase) are potential candidates for further investigation.

**Mouse Models**

Many mouse models exhibit both naturally occurring NTDs in various mouse strains, as well as NTDs that have been created by “knocking out” various genes. Examples of such mouse models include vacuolated lens, curly tail, splotch, undulated/patch, rachit-erata, truncate, and T(Brachyury) (Table 3). Although the mouse mutant T(Brachyury) does not commonly have spina bifida, mice homozygous for the mutation have severely kinked neural tubes in the caudal region and the surface ectoderm forms large fluid-filled blisters. Recent studies in humans have indicated a possible role for the human equivalent T in spina bifi-da. Most recently, double-mutant mice for the undulated/patch genes have been noted to have NTDs, although mice homozygous for either one of these recessive genes do not have NTDs, indicating digenic inheritance for the trait in this model. The equivalent human genes (when known) or the syntenic region in humans to those in the mouse provide an excellent starting point to search for genes involved in NTD for-
reported in 17 US families and 14 Dutch families and no linkage was detected.109

Mice homozygous for the undulated mutation display abnormalities along the vertebral column but do not have spina bifida as part of their phenotype. Undulated mice have a point mutation in the \textit{Pax1} gene. Heterozygous patch mice are characterized by patches of white fur, and homozygous mice die during midgestation as a result of general mesodermal deficiency. The patch phenotype is secondary to a 400 kb deletion that includes \textit{PDGFRA} and some additional loci. Although neither undulated nor patch mutant mice alone exhibit spina bifida, double-mutant mice with both copies of \textit{Pax1} mutated and only one copy of \textit{PDGFRA} present all show spina bifida as their phenotype.42

The homozygous rachiterata (rh) mutant mouse is small in size, has a short tail with distal kinks, and has a missing or abnormal axis, fused ribs, and malformations of the thoraco-lumbar region.110 In addition, these mice have 6 cervical vertebrae instead of the normal 7. The malformations of the thoraco-lumbar region reflect a primary disturbance first detectable at 11 days of gestation, due to an altered arrangement of somites.111 The \textit{rh} gene has been localized to a region of mouse chromosome 2 between the \textit{Gcg} and \textit{Hoxd} genes. This region of mouse chromosome 2 is syntenic to human 2q23-q31.

Mice homozygous for the recessive truncate (tc) gene have short or absent tails, and many mutant mice have missing sacral or lumbar vertebrae. The primary effect in truncate mice is degeneration of the notochord at 9.5 to 10 days of gestation, leading to interruption of the spine, paralysis of the hind legs, and absence of the median ventral fissure of the spinal cord.112 The \textit{tc} gene is located on mouse chromosome 6 in a region syntenic to human 2p13-p12.

The \textit{T} mutant \textit{Brachyury} was one of the earliest developmental loci to be characterized in the mouse. Because of findings in both homozygous and heterozygous mice with the mutation, investigators have long considered the locus as potentially important in humans for development of NTDs. Homozygous mice die in midgestation and display severe defects in posterior mesodermal tissues. Heterozygous mice with one remaining functional \textit{T} gene are viable, but have shortened or nonexistent tails, fusions between the gut and neural tube, localized duplications of one or both structures, and occasional malformations of the sacral vertebrae.113 Human \textit{T} has been cloned and maps to 6q27. The investigators who cloned \textit{T} studied a polymorphism they detected in intron 7 for a genetic association study in spina bifida–affected families. Evidence for a significant (\(P = .02\)) association between one of the alleles (IVS7-2) and spina bifida was detected.101

Homozygous mice for the Loop-tail (\textit{Lp}) gene have craniorachischisis, in which the neural tube remains open from the midbrain to the tail.40 Mapping studies in the mouse initially placed the \textit{Lp} gene on distal mouse chromosome 1, and recent studies using newer recombinant DNA technology have been able to further localize the \textit{Lp} gene to the region of mouse chromosome 1 syntenic to human 1q21-q23.

Danforth’s short tail (\textit{Sd}) is a semidominant mutation of the mouse that affects development of the vertebral column and the urogenital system. Heterozygous mice have shortened tails and kidney defects. Homozygous mice die at or shortly after birth, are completely tailless, and are often missing sacral and lumbar vertebrae.114 Both heterozygous and homozygous \textit{Sd} mutant mice lack a floor plate in the lumbosacral region of the spinal cord and show degenerative changes along the entire length of the notochord.115 Alterations in the notochord of \textit{Sd} homozygotes first appear between days 9.5 and 10.5 of gestation, during which time the notochord undergoes a degenerative change in the cervical and thoracic region, and formation of the notochord is never achieved in the prospective lumbosacrococaudal region.114 The proximal portion of the chromosomal region containing \textit{Sd} shows homology with human chromosome 10p.116

\textbf{Advances in Prenatal Surgery}

Despite advancement and improvement of overall patient care, little progress has been made in the postnatal surgical management of children with spina bifida. The goal of fetal surgical intervention in the treatment of meningomyelocele is to correct the structural defect at a time when significant neuronal damage has either not yet occurred or still has the potential to be reversed.17 Olutoye and Adzick17 explain that the neural damage to the exposed spinal cord in meningomyelocele secondarily results from failure of mesodermal migration, which can potentially be prevented if an adequate prenatal covering can be provided before the onset of irreversible neural damage. Other types of secondary, or late, neural damage include trauma to the exposed spinal cord during passage through the birth canal, direct abrasion of the exposed spinal cord against
the uterine wall, as well as exposure to substances in the amniotic fluid which may produce chemical injury when in contact with the unprotected neural tissues of the meningomyelocele. Therefore, coverage of the exposed spinal cord in utero could theoretically prevent these forms of secondary injury or potentially reverse any damage that has possibly occurred.

According to Olutoye and Adzick, in utero intervention to have the best possible outcome repair should be performed before the occurrence of irreversible damage. Additionally, if intervention occurs before the onset of myelination, the damaged spinal cord may retain the ability to undergo regenerative repair, as well as be protected from further injury. Preliminary results with human fetal meningomyelocele repair suggest that resolution of hydrocephalus and Arnold-Chiari type II malformation, two anomalies commonly associated with spina bifida, may be an unexpected benefit of in utero repair, especially when a long gestation period follows the repair.

Olutoye and Adzick suggest in utero meningomyelocele repair be performed on fetuses before the end of the 24th week of gestation, thus taking advantage of the healing properties of younger fetuses, potentially preserving the regenerative potential of unmyelinated spinal cord, eliminating third trimester damage to the spinal cord, and intervening early in the course of progressive hydrocephaly and Arnold-Chiari malformation often seen in fetuses with meningomyelocele.

Fetal surgery for meningomyelocele is performed after administering maternal general and epidural anesthesia, providing adequate fetal anesthesia and uterine relaxation for the fetal surgery. A low transverse maternal laparotomy is made to expose the uterus and the back of the fetus is then positioned to expose the meningomyelocele while keeping the fetus within the uterus. The cystic membrane of the meningomyelocele is carefully excised, the attachments of the meninges are detached, and the spinal cord is allowed to fall back into the vertebral canal. Because of the concern for tethering of the spinal cord to the overlying skin closure, an acellular dermal graft is placed between the spinal cord and the skin, and the bilateral skin flaps are subsequently elevated and closed over the patch.

Postoperative follow-up includes an ultrasonography twice a week in order to assess fetal well-being, as well as a fetal magnetic resonance imaging performed every 3 weeks to further evaluate brain and spinal cord development. Pregnancy is continued until 36 weeks of gestation, at which time an elective cesarean section is planned.

Olutoye and Adzick report that their group was the first to document improved neurologic outcome after in utero fetal meningomyelocele repair. They performed an open fetal surgical repair of a large meningomyelocele in a 23-week-gestation fetus that was subsequently delivered by cesarean section after the onset of preterm labor at 30 weeks of gestation. Postnatally they report that there was no hydrocephalus, and hindbrain herniation was no longer present. The neurologic outcome suggested the effectiveness of in utero fetal meningomyelocele repair in preventing further damage to the exposed neural tissue and possibly allowing restoration of function or regeneration because of the plasticity of the fetal nervous system.

Summary

NTDs, resulting from failure of the neural tube to close during the fourth week of embryogenesis, are the most common severely disabling birth defects in the United States, with a frequency of approximately 1 of every 2000 births. Neural tube malformations involving the spinal cord and vertebral arches are referred to as spina bifida, with severe types of spina bifida involving protrusion of the spinal cord and/or meninges through a defect in the vertebral arch. Depending on the level of the lesion, interruption of the spinal cord at the site of the spina bifida defect causes paralysis of the legs, incontinence of urine and feces, anesthesis of the skin, and abnormalities of the hips, knees, and feet. Two additional abnormalities often seen in children with spina bifida include hydrocephalus and the Arnold-Chiari type II malformation. Despite the physical and particular learning disabilities children with spina bifida must cope with, participation in individualized educational programs can allow these children to develop skills necessary for autonomy in adulthood.

Advances in research to uncover the molecular basis of NTDs is enhanced by knowledge of the link between the environmental and genetic factors involved in the etiology of NTDs. The most recent development in NTD research for disease-causing genes is the discovery of a genetic link to the most well-known environmental cause of neural tube malformation, folate deficiency in pregnant women. Nearly a decade ago, periconceptional folic acid supplementation was proven to decrease both the recurrence and occurrence of NTDs. The study of folate and its association with

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NTDs is an ongoing endeavor that has led to numerous studies of different genes involved in the folate metabolism pathway, including the most commonly studied thermolabile mutation (C677T) in the MTHFR gene. An additional focus for NTD research involves mouse models that exhibit both naturally occurring NTDs, as well as those created by experimental design. We hope the search for genes involved in the risk and/or development of NTDs will lead to the development of strategies for prevention and treatment.

The most recent achievement in treatment of NTDs involves the repair of meningomyelocele through advancements in fetal surgery. Convincing experimental evidence exists that in utero repair preserves neurologic function, as well as resolving the hydrocephalus and Arnold-Chiari malformation that often accompany meningomyelocele defects. However, follow-up is needed to completely evaluate long-term neurologic function and overall improved quality of life. And in the words of Olutoye and Adzick,17 "until the benefits of fetal [meningomyelocele] repair are carefully elucidated, weighed against maternal and fetal risks, and compared to conventional postnatal therapy, this procedure should be restricted to a few centers that are committed (clinically and experimentally) to investigating these issues."

References


91. Morrison K, Papaetrou C, Attwood J, Hol F, Lynch SA, Sampath A, et al. Genetic mapping of the human homologue (T) of mouse T(Brachyury) and a search for allele associa-