10 Spinal Dysraphism: The Search For Magic

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10.1 INTRODUCTION: WHY MAGIC?

Although pediatric neurosurgery is relatively young as a formal subspecialty of general neurosurgery (the first meeting of the Section of Pediatric Neurological Surgery was held in 1972 and the American Society of Pediatric Neurosurgery first met in 1978), it has been practiced for millennia. Trephined pediatric skulls were excavated in Peru and at other ancient sites. The father of neurosurgery, Sir Victor Horsley, performed his first epilepsy surgery on a child in 1886. Harvey Cushing wrote extensively about the unique disorders of childhood. Many other notables followed these icons, ensuring the momentum for further progress and refinement in the surgical care of children with disorders of the nervous system.

Congenital spinal nervous system abnormalities continue to be the mainstays and also the pitfalls of pediatric neurosurgery. Paralysis, incontinence, obesity, endocrinopathy, hydrocephalus, short stature, social stigmata, and shortened lifespans are still the norms for children with open neural tube defects (NTDs). The number of children born with myelomeningocele has decreased over the past several decades and 3 of every 10,000 children born in the U.S. are handicapped by open spinal NTDs. Additionally, improved imaging techniques have diagnosed even more children who suffer from spinal cord dysfunctions secondary to closed dysraphisms. The future treatment objectives are clear: congenital spinal defects must be prevented or their neurological sequelae must be cured. Imagine that a pill or procedure could
prevent or cure neurological deficits. Attaining such a goal often seems impossible. It would seem to require magic — but what if magic did exist?

10.2 WHY MAGIC IS NEEDED: CAUSATIVE FACTORS OF NTDs

Current hypotheses suggest that NTDs are caused by complex interactions between extrinsic (drugs, environmental toxins, temperature, etc.) and intrinsic (genetic, metabolic, etc.) variables. Clinical and epidemiological studies in humans have implicated maternal illnesses, medications, environmental toxins, and dietary factors such as folic acid that play causative or at least contributing roles in NTD development. Evidence that mutant genes cause NTDs has been supported by epidemiological studies revealing an increased incidence of NTDs in certain families. In familial cases, the trait for a NTD is semi-dominant, with apparent maternal inheritance. Thus, NTDs represent examples of complex genetic disorders in which genes and the environment interact through an unknown relationship.

Extrinsic factors causally related to NTDs have been studied extensively. Vitamins in general and folates in particular have been shown to significantly reduce children’s risks of NTD, particularly when siblings have NTDs. The protective mechanism of folate is unknown. Mothers have not been shown to be folate-deficient or have defective intestinal uptakes of folate. Studies of mutant enzymes in the folate metabolic pathway, particularly, methylenetetrahydrofolate reductase (MTHFR), suggest a possible association with NTDs. However, Speer et al. could not demonstrate MTHFR as a major risk factor. Other cellular interactions, such as cellular transport mechanisms, are currently under investigation. Folate can act as a methyl donor, permanently altering gene function via an epigenetic mechanism or interfering with a metabolic pathway such as homocysteine conversion to methionine. Although folic acid is irrelevant to the predominant basic mechanism of action of folate, supplementation with folic acid has reduced the risk of NTDs worldwide.

Other teratogenic agents and maternal diseases have been identified as causal factors for NTD development. Maternal diabetics have greater risks of having children with diabetic embroyopathies consisting of NTD and other organ system anomalies. Epileptic mothers using valproic acid as an anticonvulsant have a 1 to 2% risk. Those exposed to carbamazepine face approximately 0.5% risk of having children with NTDs. Obesity has been associated with increased risk of having a child born with a NTD. A twofold increase in NTD incidence was also found in obese versus non-obese mothers, regardless of use of vitamin, folate, and other nutritional supplements. Febrile illnesses and hyperthermia produced by the use of a sauna or hot tub early in pregnancy have been also suggested as causes of NTDs. The exact risk of occurrence due to maternal hyperthermia is not known.

Although strongly implicated, the specific genetic factors that cause NTD are not known. It is proposed that many different genes are involved in neural tube development. Some genes may confer strong genetic components and others may...
only exert minimal direct effects or require interaction with other genes. Environmental factors may act as triggers to genetic susceptibility.

Several lines of evidence point to a genetic component. Empiric studies have shown that the recurrence risk for NTD is greatest among first-degree relatives of an affected patient and decreases for more distant relatives. The recurrence risk for siblings of an affected patient is 2 to 5%, representing a 25- to 50-fold increase in recurrence risk compared to the general population. Techniques for identification of specific genes are based on identifying populations at high risk, such as twins, investigating the recurrence risks of NTD, and identifying mutated genes.

Mouse mutants have provided many of the genes investigated as candidates for human NTDs. More than 40 mouse species have been described, and the specific gene identified in only 6 species. The six well-known mutations are splotch (Sp), extra toes (Xt), short tails (T), patch (Ph), and targeted mutations in apolipoprotein B (ApoB) and Hox-a1. These mouse mutants provided clues to the embryopathies of NTDs and identified potential candidate genes for human investigation. For example, the Pax-3 mutation in splotch mice mirrored the mutated Pax-3 human homologue in Waardenburg’s syndrome. Furthermore, several Waardenburg’s patients have been reported to have spina bifida. Brachyury, when mutated, is responsible for short-tailed mice, and has been shown to have an association with human spina bifida. Greig’s cephalopolysyndactyly corresponds to mouse Xt, with patients revealing mutations in the Gli3 gene. Pax-3, brachyury, and Gli3 have not been shown to be major candidates for human NTDs.

10.3 WHERE MAGIC HAPPENS: DEVELOPMENT OF THE EMBRYO

Normal nervous system development of an embryo requires proper formation of embryonic axes. Determination of dorsoventral (DV) and anteroposterior (AP) domains during gastrulation appears critical for normal neural development. Axis patterning is reliant upon positional signals that provide DV and AP specifications. Furthermore, positional signals appear essential to neural tube induction and patterning.

Early embryonic axis determination is dependent on specification of anterior axial mesoderm followed by posterior axial mesodermal induction. A specified group of cells (organizers) are known to function to organize the AP domains of the embryonic axis. Several genes (brachyury, goosecoid, noggin, XLIM-1, Not-1) and diffusible morphogens (retinoids, activins, fibroblast growth factors) appear to be important in the regulation of organizer activity, specifically in posterior development of the axis. The anterior axial mesoderm (chordamesoderm) induces competent ectoderm to form archencephalic structures (telencephalon, diencephalon, optic rudiment). The posterior mesoderm (notochord) induces competent ectoderm to form the deuterencephalon (metencephalon, myelencephalon, cerebellum) and spinal cord. Induced ectoderm forms the brain, hindbrain, and spinal cord by the process of neurulation.
Neurulation follows two stages: primary and secondary. Primary neurulation begins after gastrulation when the primitive ectoderm is induced by the axial mesoderm to form a neural plate. The neural plate undergoes further elevation, folding, and fusion to form the neural tube. Neural crest cells migrate from the dorsal aspect of the neural tube. Primary neurulation forms all functional levels of the brain and spinal cord to the second sacral level in humans.

The caudal elements of the spinal cord, conus medullaris and filum terminale, are formed by secondary neurulation, which begins at a transitional zone where the dorsally located primary neural tube overlaps the more ventral mesenchymal cells of the tail bud in the future lumbosacral area. In this overlap zone, randomly arranged mesenchymal cells condense to form the medullary cord. Radially oriented peripheral cells surround a cellular central core in the medullary cord. Cavitation occurs centrally, forming multiple lumina that coalesce to form a secondary neural tube.

The source of secondary neural tube cells is under scrutiny. Recent evidence in chick embryos suggests that cells may migrate from more rostral neural plates to attain their proper positions in the secondary neural tubes. Normal caudal spinal cord patterning in humans has been described and abnormal patterning has been demonstrated in dysraphic states. Aberrant positional identity of caudal spinal cord cells may be a consequence of disrupted positional signals, faulty differentiation, or improper migration. Governing factors in the caudal neural tube pattern such as the brachyury and Pax-3 patterning genes have not been identified as major factors in spinal dysraphism.

10.4 MAGIC PILLS

Exciting and provocative evidence demonstrates that some manifestations of NTDs are preventable or reversible at any one of numerous steps along the pathway from preconception to childhood, and possibly even into adulthood. Several different therapeutic interventions (or “magic pills”) may be developed to treat the remaining types of NTDs. These pills may target genetic loci, proteins, or any of several metabolites involved in NTD development.

We now understand a great deal about the development of the neural tube, and are quickly approaching a more complete genetic characterization of the process. Ideally, NTDs could be detected early enough in development to target the defects before any permanent manifestations occurred. The epidemiological studies described definitively implicate maternal risk factors as well as inheritable and/or acquired genetic influences that may be targeted. The combination of genetic, epigenetic, and environmental factors offers numerous targets for interventions.

Preconception would be the optimal time for prevention. Mothers with modifiable risk factors should be identified and counseled. Perhaps one of the most remarkable advances in NTD treatment has been the introduction of periconceptional folic acid supplementation for the prevention of myelodysplasias. Whether taken in pill form or supplemented in dietary flour, this simple and inexpensive measure has cut the incidence and devastating sequelae of myelomeningocele by more than half. Despite this extraordinary achievement, it is still a challenge to prevent this unfortunate disorder of aberrant neural tube closure.

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Other maternal risk factors that may prove important include good control of diabetes, reduction of obesity and infections, vitamin supplementation (folate, inositol, and vitamin B₁₂), and avoidance of over-heated environments like saunas. Additionally, mothers taking valproate and carbamazapine antiepileptic medications should discontinue use or take other medications if possible to eliminate the increased risk.

It may be possible in some cases to identify mothers with inheritable genetic predispositions and counsel them during the preconception period in preparation for possible treatment during pregnancy. Several possible medications could be developed to provide genetic targeting during early fetal development. Tools for targeting candidate genes at the DNA, RNA, or protein level are all plausible possibilities. These tools could target defects in genes involved in proper neural tube patterning, folate-dependent and -independent mechanisms, or healing mechanisms. The next decade certainly will see attempts at in vitro correction of genetic defects during the blastocyst stage or manipulation of these genes in utero via delivery systems like viral vectors.

Several studies with animal models have elucidated some of the genes involved in the induction of proper neural tube development, for example, Wnt-1, Gnot1 (a notochord family homeobox gene), HOX-1, and activin.⁵⁰,⁵⁶,⁶⁰ Activin and retinoic acid regulate Gnot1 expression prior to gastrulation. The neural tube-inducing properties of sonic and bone morphogenic protein genes are also under intense investigation. The Sp mouse model has defects in neural tube closure due to mutations in the Pax-3 paired box gene.⁴⁴,⁴⁵ When genes are deleted or mutated, the fetal cells

![Figure 10.1 The magic phases of spinal dysraphism.](image-url)
may be transfected in utero with viral vectors expressing the normal gene. Alternatively, embryonic stem cell lines with normal genes may be introduced into target embryos by blastocyst injection, producing chimeras expressing enough of the normal gene to ameliorate the defective phenotype. Interestingly, folate, the earliest magic pill, has been shown to prevent NTD in the Sp and other mouse models with mutations in Cart1 and crooked tail genes.67,84,85

Hyperhomocysteinemia is another risk factor linked to increased risk of NTD that may be amenable to a genetic tool. The condition appears to be due to homozygosity of a thermolabile MTHFR deficiency.20 Genetic therapy could provide a solution. Currently available viral vectors could be designed to transfect fetal cells with the normal MTHFR gene. Hyperhomocysteinemia may also be due to reduced folate-dependent homocysteine remethylation, which provides another interesting mechanism for treating NTD.

Cytosine methylation on CpG dinucleotides of genomic DNA is one of many forms of DNA modifications that help maintain stability of numerous regions of genomic DNA.86 These heritable CpG methylation sites may be altered in early embryogenesis, but appear to remain stable with high fidelity afterward.87 This form of DNA methylation depends on the synthesis of S-adenosylmethionine, which requires methyl donors and cofactors like folate, vitamin B12, choline chloride, and anhydrous betaine.88

Maternal nutrition may affect fetal phenotype via DNA methylation. The areas of methylation that change during embryogenesis are at transposable element insertion sites in the genome that underlie epigenetic-induced phenotypic variability.89 Transient exposure to methyl donors in utero has been demonstrated to shift an epigenotype via CpG methylation of genomic DNA in mice.19 This experimentally altered phenotype persisted into adulthood. It is hypothesized that such a mechanism may underlie the corrected NTD phenotype in folate supplementation. Other methyl donors may also serve as magic pills.

Another compound that prevented folate resistance NTD in the curly tail mouse and recently in humans is inositol.90,91 The mechanism may occur via upregulation of the retinoic acid receptor beta.91,92 Inositol is also important in glucose metabolism and may play a role in hyperglycemic or obesity-related causes of NTD. All these therapeutic measures are meant to prevent or correct defects early enough in development to prevent NTDs. However, efforts to correct defects are still needed. Most forms of what we can designate as “magic repairs” are applied during intrauterine development or after birth.

10.5 MAGIC REPAIRS

In a typical scenario, a child born with a NTD undergoes repair of the defect in the first few days after birth (as with myelomeningocele) or when neurological deterioration or substantial neurological risk is determined (as with closed dysraphism). Both paradigms are designed to minimize further risk, prevent progressive functional loss, and possibly reverse neurological deterioration. Clearly, in the case of an open NTD, reversal of paralysis or sacral dysfunction is not expected or attained. Novel repair strategies should be aimed at restoration of neurological function.
10.5.1 Fetal Surgery

Recent evidence suggests that the neurological deterioration associated with open NTDs may have resulted from progressive intrauterine injury alone or in concert with the primary defect of neurulation. For example, fetal ultrasonography revealed that human fetuses with myelomeningoceles retained lower extremity movements early in gestation and that the movements were lost by term. These data and maternal reports that describe losses of fetal movements suggest that an event occurring during gestation damaged fetal function.

In the event of intrauterine injury, intrauterine intervention such as a surgical repair may protect against progressive neurological deterioration. Animal models designed with spina bifida were tested after intrauterine repair. Neurological function was preserved in repaired animals. This result led to intrauterine repairs of open, exposed spinal cords in humans.

To determine the outcomes of fetal myelomeningocele repairs, the National Institute of Child Health and Human Development (NICHD) sponsored the Management of Myelomeningocele Study (MOMS), a continuing clinical trial [http://www.nichd.nih.gov]. Parameters undergoing study include optimal timing, neurological recovery, and effects of repairs on associated hydrocephalus and Chiari II malformations. The study is comparing two approaches to the treatment of babies with spina bifida: surgery before birth (prenatal surgery) and the standard closure surgery after birth (postnatal surgery). Preliminary results of human surgery show failure to preserve fetal neurological function. Furthermore, when it appeared that spinal cord function was present to a degree, it was less than predicted based on data from the animal models. Improvements in the degree of hindbrain herniation noted in the associated Chiari II malformation have also been demonstrated. Additionally, a reduction in the need for CSF shunting for hydrocephalus has been reported.

Reported complications of fetal myelomeningocele surgery have been few; the most common complication is preterm delivery. Major complications of intrauterine intervention such as maternal death from uterine rupture have been reported for other types of fetal surgery. No uterine rupture resulting in maternal or fetal demise has been reported to date for fetal myelomeningocele repair. Technical advancements, such as less invasive endoscopic procedures, have been proposed to avert this severe complication.

One key to predicting optimal outcomes of novel fetal surgery treatments for myelomeningocele is understanding the structure of the placode. If the placode retains normal patterning and is simply un-neurulated, a repair may be effective in preventing secondary injury. There are mixed reports on whether placodes are normal in animal models. Similar controversies surround human studies. Meuli et al. characterized the human placode as having partial loss of tissue, containing hemorrhages and abrasions, while preserving developed elements of dorsal and ventral parts of the spinal cord with nerve roots and ganglia. The abnormalities were attributed to intrauterine injury.

Conversely, George and Cummings characterized the placode as having abnormal patterning along the dorsoventral and rostrocaudal axes indicative of a change...
in pattern determination and a paucity of maturing neurons with evidence of significant inflammatory infiltrate, gliosis, and fibrosis consistent with secondary injury. These data suggest that the myelomeningocele placode shows abnormal development along with evidence of injury.

Reexamination of the animal model is needed to help clarify this controversy. George and Fuh made several observations in a review. Two definitions of NTDs were used to describe the surgical models: spina bifida or spina bifida-like and surgical NTDs. All mammals except mice had spina bifida lesions in which the skin, muscle, lamina, and dura were opened, but the spinal cord itself was not disturbed. Surgical NTDs were developed in avian species and mice; the dorsal elements of the spinal cord were opened and splayed apart, and exposed the central elements of the spinal cord to the surrounding environment. The surgical models uncovered three mechanisms of injury:

1. Toxicity of the amniotic fluid
2. Direct intrauterine trauma
3. Developmental and growth distortion from laminectomy defect

Timing of lesions was critical. Spontaneous healing resulted if lesions occurred early in gestation instead of later. Subsequent functional outcomes were virtually indistinguishable between groups lesioned early in gestation and spontaneously healed and repaired fetuses lesioned later in gestation. Last, the surgical animal models used were not the products of abnormal primary neurulation, and could not directly address questions concerning the placode. These surgical models represent a reopening mechanism of a closed neural tube that has not been shown to appear in humans, but was reported in curtailed mouse mutants.

The future of fetal surgery may rest in uncovering the mechanisms of fetal healing and directly reconstituting the spinal cord. In the study of fetal wounds, healing was demonstrated to occur rapidly and without scarring. The exact mechanisms of fetal scarless healing remain unknown. However, transforming growth factor-beta and hyaluronic acid-rich wound matrix play pivotal roles in scarless repair.

The mechanism of annealing or healing that can lead to protection of the neural tube has also not been defined. The fusion of reapproximated dorsal neural elements in chicks has been suggested. A preliminary study in our laboratory utilizing surgical NTDs in chicks and adding inhibitors of primary neurulation failed to prevent reclosure of the neural tube (unpublished data). Therefore, reclosure in chicks does not appear to be a recapitulation of primary neurulation. The underlying molecular and cellular mechanisms that regulated the repair remain unclear, but the ability of spinal cord cells to proliferate appeared important. These data suggest that fetal interventions should be targeted at reinstituting mechanisms of fetal healing that were turned off after a critical developmental phase.

10.5.2 Spinal Cord Regeneration

Current work on restoration of spinal cord function has focused on regeneration after a spinal cord injury. If the precept from the fetal surgery is true, that the
neurological sequelae in open NTDs are caused by intrauterine injuries, restoration of cord function should be attainable. In fact, the majority of research has revealed that an injured spinal cord can be restored by reconstituting or reestablishing molecular or cellular developmental mechanisms. Therefore, the developing spinal cord appears to be the ideal substrate for regeneration of specific cell types and functional connections as long as the milieu can be properly manipulated.

Paramount for the regeneration of the spinal cord is that the neuron becomes “regeneration-capable” — it can restore the ability to demonstrate axonal growth and proper targeting. A number of genes have been shown to be constitutively expressed or upregulated in response to axonal growth. They have been termed “regeneration-associated genes” and their products include transcription factors such as c-jun, cytoskeleton components such as alpha tubulin, cytoplasmic growth cone proteins such as GAP-43 and CAP-23, and cell adhesion molecules such as NCAM and L1 that are important for growth cone guidance.

The rate-limiting factor impacting regeneration is the inhibitory environment of the mature CNS. CNS inhibition to axonal growth is broadly divided into nonpermissive factors related to myelin and the inhibitory nature of the gliotic scar. Proteins identified in CNS myelin (NI-35 and NI-250) have been shown to function as neurite inhibitory factors. At the injury site, dead cells, inflammation, and degraded tissue are present. They contain reactive astrocytes, microglia, oligodendrocytes, and meningeal cells that form gliotic scars that function as three-dimensional barriers to axonal growth.

As noted earlier, George and Cummings demonstrated that the myelomeningocele placode may have abnormal patterning along the dorsoventral and rostrocaudal axes. This finding is indicative of a change in pattern determination, along with a paucity of maturing neurons with evidence of significant inflammatory infiltrate, gliosis, and fibrosis consistent with secondary injury. The impact that aberrant development plays on the ability of the injured placode to regenerate and overcome the inhibitory environment is unclear and remains a goal of future research.

Regenerative strategies in spinal cord injury include administration of trophic factors, gene therapy, and cell transplantation. Intrathecal administration of trophic factors such as neurotropin, nerve growth factor and glial-derived neurotrophic factor upregulated growth cone proteins such as GAP-43 and CAP-23, propagated axonal regrowth across an area of crush injury, and established functional connections. Interestingly, the administration of folate has been reported to assist in regenerating axons in a spinal cord injury model via intraperitoneal administration (personal communication). The mechanism of folate-assisted regeneration remains unknown.

Gene therapy strategies provide a way for longer lasting delivery of important trophic factors. Trophic genes can be supplied ex vivo to an injured spinal cord by inserting genetically altered cells that produce trophic factors. Another method is applied in vivo: the neurotrophic gene is transfected into the native spinal cord, usually via a viral vector. Trophic factors listed above also serve as candidates for gene therapy. Other classes of gene candidates are endogenous receptors or morphogens important in embryonic development. For example, retinoic acid (RA) is important in embryonic neural development and has been shown to stimulate embryonic neurite outgrowth. RA administration failed to induce neurite growth in an injured
adult spinal cord, presumably due to the lack of retinoic acid receptor-beta 2 (RAR β2) upregulation. However, when the RAR β2 is upregulated, neurite outgrowth can occur. Transfection into the adult spinal cord of RAR β2 alone was shown to stimulate neurite outgrowth. Therefore, reinstitution of developmental mechanisms may be another methodology of cord regeneration.

Cellular transplantation strategies are aimed at circumventing the inhibitory surround created by the gliotic scar. Candidates for transplantation are neural stem cells and fetal cells that have the potential to develop into mature neurons or glia and restore function by replacing or repairing axons and synaptic relays. Mature cells such as Schwann cells or olfactory ensheathing cells also provide neurotrophic support and myelination, thereby enhancing the regenerative environment. How the myelomeningocele placode would respond to cellular transplantation remains unclear. The lack of understanding of cell connectivity and patterning and the way that environment responds to injury makes outcomes unpredictable, but unveils a focal point for future study.

A final challenge to spinal cord regeneration of a NTD is that most of the studies examined models of acute injury. Spinal cord dysfunction secondary in the congenital setting is more likely to be chronic in nature. An important consideration in studies of chronic injury is the survival of the injured neurons. Reports indicate that 25 to 50% of neurons die as early as 4 weeks postaxotomy, while the remaining cells become atrophic. There is some evidence that trophic factors and fetal cell transplants can enhance survival, even if applied 1 year after injury. Since many patients with open and closed defects will present with neurological dysfunction within this time frame, attempts at spinal cord regeneration remain viable techniques to pursue.

10.6 SHOULD WE BELIEVE IN MAGIC?

The short answer is “yes.” Recent advances in genomics, proteomics, developmental cell biology, biochemistry, embryology, neurobiology and neuroimaging have created the potential for a “golden age” in the cure of NTDs. Until then, NTDs remain physically debilitating and are socioeconomic burdens. The time to advance neurosurgical management from supportive to restorative is now. It will be like magic!

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