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Research Article

Spina Bifida and Neural Tube Defects J: Embryology, Genetics, Epidemiology, Management, and Therapeutic Advances

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Abstract. Spina bifida, a neural tube defect arising from incomplete closure of the developing spinal column, is the most common congenital anomaly of the central nervous system and a leading cause of lifelong physical disability. Despite decades of investigation, its pathogenesis remains incompletely characterized; however, substantial progress has been made in defining its genetic determinants and the molecular pathways governing neural tube development and closure. Parallel advances in clinical management have transformed patient care, with treatment evolving from conventional postnatal surgical repair to fetoscopic and open intrauterine correction, now regarded as a standard-of-care option for eligible candidates. In this review, we synthesize current knowledge of the classification,

embryology, genetics, and epidemiology of spina bifida; examine its pathophysiology and associated complications; and survey established and evolving treatment approaches. We conclude by highlighting the CuRe Trial, the first FDA-approved clinical trial of stem cell therapy administered at the time of intrauterine repair, whose recently published phase 1 results suggest a potential paradigm shift in the management of this condition.

Keywords: spina bifida; myelomeningocele; neural tube defects; neurulation; folate; fetal surgery; mesenchymal stem cells; Chiari II malformation

INTRODUCTION

As the most prevalent congenital anomaly of the central nervous system, spina bifida (SB) is compatible with survival but frequently results in disability that persists throughout the patient's life. The spectrum of SB varies in severity: myelomeningocele (MMC), its most severe manifestation, involves herniation of the spinal cord through a vertebral defect, with neural elements exposed within a fluid-filled sac; by contrast, closed spina bifida represents a milder subtype generally associated with more favorable outcomes than open SB (**Copp et al., 2015**). Epidemiologically, contemporary data indicate a U.S. birth prevalence of approximately 3.63 cases per 10,000 live births, compared with a global estimate of 18.6 per 10,000 birth outcomes a figure that, as detailed in Section 3, counts terminated and stillborn NTD-affected pregnancies alongside live births (**Blencowe et al., 2018**). Although mortality among liveborn infants with SB has fallen to approximately 8%, this figure remains more than tenfold the baseline mortality rate across all U.S. births (**Adzick et al., 2011; Afman et al., 2005**). Survivors commonly face motor deficits, bladder and bowel dysfunction, and a range of neurological complications. Among these, Arnold–Chiari II malformation caudal displacement of the cerebellar tonsils and vermis occurs frequently in SB and can compromise motor, cranial nerve, and cognitive function, collectively diminishing quality of life for both patients and their families.

Despite substantial gains in knowledge over the past several decades, fundamental questions regarding the mechanisms driving NTD formation, strategies for prevention, and options for definitive cure remain unresolved. Etiology and pathogenesis are now understood to reflect a complex interplay among genetic, maternal, and environmental influences (**Akar et al., 2000; Alahmari, 2026**). Heritable factors are estimated to account for roughly 60–70% of NTD risk, motivating continued efforts to identify novel susceptibility loci in human populations (**Boyle et al., 2017**). The scale of this genetic complexity is reflected in murine models, where more than 240 mutant alleles and strains have been linked to NTD phenotypes (**Harris & Juriloff, 2010**).

Here, we review the classification, epidemiology, and genetic and embryological foundations of SB and NTDs; introduce associated complications and the role of folate metabolism; provide an overview of pathophysiology and surgical management; and survey historical and contemporary advances in stem cell-based therapeutic strategies.

Embryology of SB

The neural tube is a transient embryonic structure that gives rise to the central nervous system, encompassing both brain and spinal cord. This process unfolds between days 17 and 28 post-fertilization in humans (**Alahmari, 2026**) and depends on a coordinated series of cellular events apoptosis, neural crest cell migration, neuroepithelial proliferation, apical constriction driven by cytoskeletal microfilaments, and bending at dorsolateral hinge points (**Avagliano et al., 2019**). Disruption of any of these events during embryogenesis can result in failed closure and a consequent NTD. Broadly, neural tube closure proceeds through folding, elevation, apposition, and fusion along the dorsal midline, separating neural from non-neural tissue. This overarching process, termed neurulation, occurs via two distinct mechanisms: primary and secondary neurulation (**Avagliano et al., 2019**).

Primary neurulation generates the brain and the spinal cord spanning from the medulla to the mid-lumbar enlargement, driving the folding and midline fusion of the neural plate. In mouse embryos, this process begins at the junction between the cervical spine and presumptive hindbrain, with closure extending bidirectionally from that point; a second initiation site emerges at the forebrain–midbrain boundary and likewise spreads bidirectionally, while a third site arises at the rostral forebrain. Convergence of closure from all three sites completes cranial and spinal neurulation, sealing the anterior, posterior, and hindbrain neuropores (**Avagliano et al., 2019**). Human neurulation is thought to mirror this pattern with several notable distinctions (**Azzarà et al., 2021**). Whether a second closure site ("Closure 2") truly exists in humans okremains debated, and cranial closure may instead proceed as a continuous progression from Closure 1 to Closure 3 a discrepancy that may reflect population-level variation in Closure 2 positioning, paralleling strain-dependent variation in mice, or may indicate that primates have lost the need for a discrete Closure 2 site given their comparatively smaller brain size at this developmental stage (**Beaumont., et al 2019**).

Errors during primary neurulation produce NTDs, including SB, that vary according to the location and extent of unfused neural tube, reflecting the segmental nature of closure. Because complete neurulation requires successful convergence of all three sites, failure at the initial site produces defects spanning the midbrain to the lower spine craniorachischisis, marked by absence of the brain and cranial vault alongside a continuous bony spinal defect. Anencephaly, the absence of major portions of the brain, cranium, and scalp, results specifically from failed cranial neurulation, whereas SB arises from incomplete closure at the caudal end of the neural tube (**Avagliano et al., 2019**).

Secondary neurulation, by contrast, generates the most caudal spinal cord segments the distal lumbar cord, conus medullaris, and filum terminale through canalization and retrogressive differentiation. Canalization is the caudally directed elongation of the neural tube toward the posterior neuropore: as the notochord fuses with the overlying neural epithelium, a caudal cell mass forms, within which coalescing microcysts create an ependyma-lined tubular structure that joins the neural tube above, yielding a single continuous structure (**Alahmari, 2026**). In humans, this process concludes by day 28; beginning around day 38, programmed cell death along the caudal neural tube and retrogressive differentiation eliminate most of the caudal

cell mass and shape the distal lumbar cord, conus medullaris, and filum terminale. This contrasts with mice, in which the analogous cell mass instead persists to form a tail (**Beaumont et al., 2019**).

Disrupted secondary neurulation produces a distinct spectrum of anomalies, including caudal agenesis, presacral cysts, hindgut malformations, caudal lipomas, and anorectal or genitourinary abnormalities (**Pang et al., 2020**). One such lesion, the retained medullary cord (RMC), is an elongated, spinal-cord-like structure extending to the cul-de-sac, thought to result from complete or partial arrest of secondary neurulation (**Pang, Zovickian, & Moes, 2011**); it resembles a low-lying conus but is functionally inert. Once considered rare, RMC is now recognized with increasing frequency and often co-occurs with the anomalies noted above. Affected patients typically present with urinary incontinence, recurrent urinary tract infections, neurogenic bladder, urodynamic abnormalities, and neurologic deficits (**Kim, Lee, & Wang, 2020**).

Two mechanisms have been proposed for NTD formation: primary failure of closure due to disordered cellular behavior (impaired proliferation, dysregulated apoptosis, defective collective migration), or reopening of an initially closed neural tube, potentially via breakdown of critical cell–cell adhesion junctions (**Blencowe, Kancherla, et al., 2018; Borgel et al., 2010**).

Epidemiology of SB

Since neural tube defects were first characterized, their measured prevalence and incidence have shifted over time, though the overall trajectory has been downward a trend driven largely by targeted intervention programs and expanded prenatal screening. Folic acid fortification of the food supply is credited with preventing an estimated 1,300 NTD-affected births each year in the United States that would otherwise have occurred (**Van Der Pals et al., 2007**).

Worldwide, NTD-affected pregnancy outcomes occur at a modeled rate of approximately 18.6 per 10,000 live births, though reported prevalence varies enormously by region and surveillance method from under 1 to over 100 per 10,000 births across published estimates. This wide range partly reflects a genuine difference in what is being counted: because roughly half of NTD-affected pregnancies worldwide end in elective termination or stillbirth, estimates that (like Blencowe et al.'s) include all affected pregnancy outcomes will run substantially higher than estimates restricted to live-born prevalence alone. In many low- and middle-income countries, limited access to prenatal diagnosis and incomplete birth-defect surveillance likely lead to further underestimation of the true burden **(Blencowe, Kancherla, et al., 2018).**

Geographic variation is also apparent: European data put spina bifida prevalence at 4.9 per 10,000 births, compared with a somewhat lower U.S. prevalence of 3.17 per 10,000 live births recorded between 1999 and 2007 (**Canfield et al., 2014**). Since the introduction of fortification, the U.S. rate has fallen by roughly 25%, now standing at approximately 1 case per 1,500 births (**Abdelmageed et al., 2024**).

Ethnicity also plays a role in NTD risk, with Hispanic women showing a consistently elevated rate of affected pregnancies relative to other groups. Combined international data show spina bifida prevalence of 3.8 per 10,000 live births among

Hispanic populations, versus 3.09 per 10,000 among non-Hispanic White individuals and 2.73 per 10,000 among non-Hispanic Black individuals (**Wang, Liu, Canfield, et al., 2015**). Recurrence risk in subsequent pregnancies, meanwhile, depends on multiple variables — including family history, geographic region, and the severity of the original defect. Families with one prior NTD-affected pregnancy, or a maternal history of the condition, face an elevated recurrence risk of roughly 3% to 8%; this risk climbs further with each additional affected pregnancy (**Suarez et al., 2003**).

Classification of Spinal Dysraphism

Spinal dysraphism is an umbrella term for congenital malformations arising from defective development or closure of the neural tube and its surrounding mesenchymal, osseous, and cutaneous structures; spina bifida is its most common and clinically significant form. The clinical-radiological classification introduced by Tortori-Donati, Rossi, and Cama remains the most widely used framework in both the neurosurgical and radiological literature (**Tortori-Donati, Rossi, & Cama, 2000**). It divides spinal dysraphism first by whether neural tissue is exposed to the environment (open vs. closed), and then subdivides the closed forms according to whether a subcutaneous mass is present.

Open spinal dysraphism (spina bifida aperta/cystica) is characterized by a neural placode that is not covered by skin, and is essentially always accompanied by Chiari II malformation. Myelomeningocele accounts for the substantial majority of cases and is the form most relevant to fetal surgical intervention (discussed in Section 9.4); rarer variants include myelocele (or myeloschisis, in which the flat placode lies level with the skin surface without a protruding cystic sac) and hemimyelomeningocele/hemimyelocele, seen in association with split notochord anomalies (**Dhombres, F., et al. 2025**)

Closed spinal dysraphism (spina bifida occulta) is characterized by neural tissue fully covered by skin, and is subdivided by the presence or absence of an associated subcutaneous mass:

- *With a subcutaneous mass*: lipomyelomeningocele, lipomyelocele, meningocele, and myelocystocele.
- *Without a subcutaneous mass*, comprising:

Simple dysraphic states, generally isolated anomalies of secondary neurulation: tight or thickened filum terminale, filar or intradural lipoma, persistent terminal ventricle, and dermal sinus tract. The retained medullary cord discussed in Section 2 falls within this group.

Complex dysraphic states, typically reflecting earlier, gastrulation-stage disruption and often accompanied by other malformations: split cord malformations (diastematomyelia), caudal regression/agenesis, segmental spinal dysgenesis, and disorders of notochordal integration such as neurenteric cyst(**Dhombres, F., et al. (2025)**). Table 1 summarizes this framework alongside the principal entities within each category.

Table 1. Clinical-radiological classification of spinal dysraphism

Category	Subtypes
Open spinal dysraphism (spina bifida aperta/cystica)	Myelomeningocele; myelocele/myeloschisis; hemimyelomeningocele/hemimyelocele
Closed spinal dysraphism, with subcutaneous mass	Lipomyelomeningocele; lipomyelocele; meningocele; myelocystocele
Closed spinal dysraphism, without mass — simple	Tight/thickened filum terminale; filar or intradural lipoma; persistent terminal ventricle; dermal sinus tract; retained medullary cord
Closed spinal dysraphism, without mass — complex	Diastematomyelia (split cord malformation); caudal regression/agenesis; segmental spinal dysgenesis; neurenteric cyst
Associated osseous anomalies	Hemivertebrae; butterfly vertebrae; vertebral fusion anomalies; sacral agenesis (in the context of caudal regression syndrome)

Genetics and Molecular Aspects of SB

Although family history is a recognized risk factor for SB, its recurrence does not map to a single causative locus; rather, SB is a complex, multifactorial trait arising from variants across multiple genes and loci (**Copp, Stanier, & Greene, 2013**).

1. Neural tube defects in animal models

Studies in mice have identified more than 240 mutant alleles and strains associated with NTDs, implicating pathways including planar cell polarity (PCP) signaling, apoptosis, transcriptional regulation, and canonical Wnt/β-catenin signaling (**Harris & Juriloff, 2010**). Proper apical–basal polarization underlies normal tissue morphogenesis and function. VANGL1 and VANGL2, mammalian homologs of the *Drosophila* gene *strabismus*/*Van Gogh* required for PCP in the eye, wing, and leg, are expressed in the dorsal neural tube and are essential for vertebrate morphogenesis (**Kibar, Vogan, et al., 2001**). Song and colleagues showed that PCP signaling links anterior–posterior patterning cues to left–right asymmetry in the notochord, acting upstream of leftward nodal flow (**Song et al., 2010**); accordingly, notochord cells lacking VANGL1/VANGL2 display randomized ciliary orientation, producing turbulent nodal flow and disrupted symmetry. Notably, Torban and colleagues found that only mice heterozygous for combined VANGL1 and VANGL2 mutations developed severe defects, including craniorachischisis (**Torban et al., 2008**).

PCP establishment also depends on the Wnt/Frizzled-PCP cascade. In *Drosophila*, this involves the cadherin Flamingo, the receptor Frizzled (Fz), and cytoplasmic effectors Dishevelled (Dsh), Diego (Dgo), and Prickle (Pk); disrupting any of these causes loss of polarity and cytoskeletal disorganization (**Chen et al., 2018**). Their mammalian orthologs CELSR, DVL, FZD, and Prickle have each been implicated in NTD etiology (**Robinson et al., 2012**). Tissue-specific, non-core PCP effectors matter too: FUZ, involved in directional cell movement, is required for neural tube

closure in mice and humans, and *Fuzzy*-knockout mice develop NTDs, prompting the proposal that FUZ mutations may underlie some human cases (**Gray et al., 2009**). Humphries and colleagues extended this work by introducing mammalian PCP mutations into *Drosophila*, observing distinct phenotypic and functional abnormalities that further support a causal PCP–NTD relationship (**Humphries, Narang, & Mlodzik, 2020**).

Other murine models implicate transcriptional and apoptotic regulators. Foxc2 (MFH-1) is proposed to mediate skeletogenesis in neural crest- and mesoderm-derived cells (**Copp, Stanier, & Greene, 2013**), while mutations in *Traf4*, an apoptosis-related gene, impair nucleosome assembly protein binding to condensing chromatin during apoptosis and disrupt neuronal proliferation (**Régnier et al., 2002**).

Finally, canonical Wnt/β-catenin pathway disruption has been demonstrated in murine SB models and implicated in human disease. LRP6 (low-density lipoprotein receptor-related protein 6), a regulator of skeletal growth and remodeling within this pathway, contributes to axial skeleton and neural tube development (**Kokubu et al., 2004**). Lei and colleagues identified multiple LRP6 single-nucleotide variants that predispose to NTDs.

The Mediator complex (MED) similarly regulates Wnt signaling and has been linked to neural tube closure (**Rocha, Bleiss, & Schrewe, 2010**), with CRISPR/Cas9-generated knock-in mouse models providing strong evidence linking functional MED variants to NTD etiology (**Tian et al., 2021**). Canonical Wnt signaling further intersects with PAX3, regulating and being regulated by it, and PAX3 mutations disrupt neural tube closure in a manner preventable by folic acid (**Sudiwala et al., 2019**); recent evidence shows that gain-of-function β-catenin and loss-of-function PAX3 mutations interact additively to increase NTD risk (**Palmer et al., 2021**).

2. Human genetics

The pattern of SB inheritance in humans is gene-dependent. Autosomal dominant transmission has been documented at several loci: VANGL1, VANGL2, FUZ, CELSR1, and TBXT among them. Even so, identifying these individual genes tells only part of the story: an expanding body of evidence now supports an omnigenic model in which human spina bifida results from the cumulative effect of numerous genetic variants acting together with environmental exposures (**Boyle, Li, & Pritchard, 2017**). Several variants in VANGL1 have been documented across diverse NTD phenotypes in humans(**Kibar et al., 2007**), and a review by Marello and colleagues similarly reported a strong link between rare VANGL1 variants and NTD risk.

VANGL2 has received comparable attention: sequencing efforts by both Lei and colleagues and Kibar and colleagues, conducted in NTD-affected cohorts, implicated VANGL2 mutations as a possible predisposing factor for human NTDs (**Bartsch et al., 2012**, while separate work has proposed that indirect disruption of VANGL2 function short of outright mutation may also raise NTD risk.

Among 192 patients studied in California, a heterozygous frameshift variant in CELSR1 was associated with SB (**Lei, Zhu, Yang, et al., 2014**); because the CELSR1 protein normally partners with VANGL2 to establish cell-to-cell contact, this variant is thought to act by reducing VANGL2 recruitment to that interface (**Robinson et**

al., 2012). Separately, an Italian cohort study identified five distinct heterozygous missense mutations in FUZ that disrupt ciliogenesis, directional cell migration, or both, further implicating this gene in NTD development (**Seo et al., 2011**).

CCL2 constitutes an additional genetic risk factor for spina bifida, functioning to regulate export of monocyte chemotactic protein-1 (MCP-1) in response to interleukin-1-beta stimulation in vitro (**Jensen, Etheredge, et al., 2006**). This is notable in light of evidence linking first-trimester maternal hyperthermia to a twofold rise in spina bifida risk, together pointing toward inflammatory processes and thermal stress potentially acting via chemokine signaling as contributors to disease pathogenesis. In line with this, Jensen and colleagues identified an association between a polymorphism in the CCL2 A(-2518)G promoter and spina bifida, proposing that carriers of this variant mount a comparatively muted systemic and local response to infection (**Jensen, Etheredge, et al., 2006**).

Meningomyelocele has also been associated with a specific TBXT allelic variant, TIVS7-2 (**Shields et al., 2000**). TBXT, the product of the T gene, is essential to posterior mesoderm formation and axial development in vertebrates; subsequent work has confirmed the link between the TIVS7-2 variant and heightened SB risk, though the mechanism by which it exerts this effect has not yet been determined (**Jensen, Barbaux, et al., 2004**).

Given that folate supplementation can prevent as many as 70% of NTD cases, mutations affecting enzymes of the homocysteine-folate metabolic pathway methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS), enzymes involved in cobalamin coenzyme synthesis, and cystathionine β-synthase (CBS) have been a major focus of human genetic studies (**Greene, Stanier, & Moore, 2011**). The MTHFR R653Q single-nucleotide polymorphism has been reported in NTD cases among Irish and Italian cohorts (**Parle-McDermott et al., 2006; Brody et al., 2002**), and the combination of MTHFR 1298 A→C with 677 C→T variants has been shown to further elevate spina bifida risk (**Kirke et al., 2004**). Polymorphisms affecting MS and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) show similar patterns: work by Doolin and colleagues linked both the MS 2756A→G and MTRR 66A→G polymorphisms to increased spina bifida risk in a maternal genotype-dependent manner (**Doolin et al., 2002**). Gene-nutrient interactions add a further layer of complexity: MTHFR polymorphism paired with low maternal folate status carries greater NTD risk than either factor alone, and the MTRR 66A→G polymorphism combined with low serum B12 in both mother and child has likewise been shown to increase spina bifida risk (**Ray & Blom, 2003**).

The emergence of next-generation whole-exome sequencing (NGS) has made it possible to identify variants in candidate genes not previously connected to human SB. Loss-of-function de novo variants in SHROOM3, PAX3, GRHL3, and MYO1E have each been tied to NTD development, with proposed mechanisms spanning protein-truncating mutations and disrupted transcription factor activity (**Lemay, Guyot, et al., 2018; Lemay, Marco, Emond, et al., 2017**). More recent work has implicated rare de novo variants in RXRγ, DTX1, and COL15A1, alongside X-linked recessive variants in ARHGAP36, TKTL1, AMOT, GPR50, and NKRF, as additional contributors to NTD risk (**Lemay, Marco, Traverso, et al., 2018**). Exome sequencing has also reinforced the

connection between PCP pathway genes and both SB and craniorachischisis, with contributing genes now including CELSR1, PRICKLE1, FZD6, SCRIB, PTK7, and VANGL1, as well as the more recently identified FREM2 and DISP2 (**Azzarà et al., 2021**). Because NTDs arise from such heterogeneous, omnigenic genetic architecture, the genetic targets of clinical relevance are likely to differ from one patient to the next, making genome sequencing an increasingly essential tool for deepening our understanding of NTD genetics and advancing precision medicine approaches to SB.

Nongenetic Factors

1. Maternal factors

Maternal metabolic status also shapes NTD risk, with both diabetes and obesity independently implicated (**Anderson et al., 2005; Hendricks et al., 2001**). The teratogenicity of hyperglycemia and hyperinsulinemia is thought to stem from increased apoptosis within the neuroepithelium of the neural plate, and the resulting risk is substantial: a 2- to 10-fold increase associated with maternal diabetes and a 1.5- to 3.5-fold increase with obesity, with risk scaling alongside maternal body mass index (**Kajiwara et al. 2017**). Notably, this elevated risk is not confined to insulin-resistant (type 2) diabetes; offspring of mothers with insulin-dependent (type 1) diabetes face similar risk, underscoring the importance of prenatal counseling in this population (**Evers, de Valk, & Visser, 2004; Taylor & Davison, 2007**). Maternal nutritional deficiencies constitute a separate risk pathway: low folate, zinc, and B12 have each been implicated, though the evidence base for folate remains strongest (**Kirke et al., 2004; Suarez et al., 2003**). Alcohol and tobacco use, and caffeine consumption, have likewise been proposed as contributing risk factors (**Grewal et al., 2008; Schmidt et al., 2009**). Maternal hyperthermia whether febrile or environmental in origin — has similarly been hypothesized to exert teratogenic effects on the developing embryo; a systematic review and meta-analysis by Moretti and colleagues quantified this risk, reporting a significantly elevated odds ratio of 1.92 (95% CI: 1.61–2.29) for NTDs in pregnancies complicated by maternal hyperthermia (**Moretti et al., 2005**), with consistent risk elevation regardless of whether the heat source was fever, sauna use, or exercise.

2. Medications

Among pharmacologic contributors to NTD risk, valproate (valproic acid) is the most extensively documented; spina bifida occurring in pregnancies among women treated with valproate for seizure disorders was first reported by Robert and colleagues (**Robert E, et al.1982**)

Mechanistically, valproate is understood to act as a histone deacetylase inhibitor, disrupting the normal balance of protein acetylation and deacetylation and thereby disrupting Wnt signaling and, ultimately, neurulation itself (**Jentink, 2005**). Comparative studies examining other antiepileptic drugs including carbamazepine, phenytoin, and lamotrigine have consistently found valproate to carry the most severe risk profile for the developing fetus; because this risk is dose-dependent, current guidance favors avoiding valproate where clinically feasible or, failing that, minimizing the dose used (**Weston et al., 2016**).

Folate Metabolism

Folates comprise a family of water-soluble vitamins found naturally in fruits and leafy green vegetables. Folic acid, by contrast, is a synthetic form favored in pharmaceutical and supplement formulations owing to its chemical stability; "folate" serves as the umbrella term encompassing both natural and synthetic variants. As the most oxidized folate species, folic acid (pteroylglutamate) requires reduction before it becomes biologically active a two-step conversion, first to dihydrofolate and then to the active tetrahydrofolate form, catalyzed at each step by dihydrofolate reductase and utilized by all proliferating cells. Further modification through single-carbon addition and reduction yields 5-methyltetrahydrofolate (5-MTHF), the dominant folate species circulating in plasma.

How folate supplementation prevents NTDs is not fully understood, though a leading hypothesis holds that it corrects a disturbance in cellular growth within the neuroepithelium of the neural plate and neural fold (**Sato, 2020**). This tissue's rapid proliferation demands a correspondingly high rate of nucleic acid synthesis, a process for which 5-MTHF generated from tetrahydrofolate via the glycine cleavage system (GCS) is essential. The GCS, a core component of glycine and serine catabolism, is both a principal source of 5-MTHF (one of the few one-carbon donors available in vertebrate biosynthesis) and highly expressed within the neuroepithelium itself (**Ichinohe et al., 2004**). Folate's central metabolic role is the shuttling of single-carbon units, acting alternately as donor or acceptor to support DNA synthesis and DNA/RNA modification, functioning throughout as an essential coenzyme. Collectively, these reactions constitute folate-mediated one-carbon metabolism, an interconnected network spanning the cytosol, nucleus, and mitochondria (**Lan, Field, & Stover, 2018**).

Cellular folate uptake in mammals proceeds via folate receptors that internalize plasma 5-MTHF through endocytosis; within the cell, subsequent addition of glutamate residues (polyglutamation) traps folate intracellularly. The gene *FOLR1*, which encodes a protein mediating folate transport into neuroepithelial and neural crest cells, has been characterized in murine models, where the protein localizes predominantly to epithelial cells of the choroid plexus, kidney, ovary, and placenta; functional loss of FOLR1 produces defects spanning the cranium, neural tube, and cardiac tissue. Critically, these defects could be rescued in heterozygous embryos through folate supplementation, demonstrating that impaired 5-MTHF uptake capacity can be functionally compensated by increasing folate availability (**Zhao et al., 2001**).

A second proposed mechanism implicates epigenetic regulation, particularly DNA methylation, as a pathway through which folate supplementation protects against NTDs a plausible route given the extensive chromatin methylation characteristic of early embryogenesis (**Borgel et al., 2010**). Supporting this, murine studies have repeatedly linked methylation disruption to increased NTD rates (**Dunlevy, Burren, Chitty, et al., 2006**). Perturbations of the methylation cycle alter the balance between S-adenosylmethionine (SAM), the principal methyl donor, and

S-adenosylhomocysteine (SAH), which is generated when SAM loses its methyl group (**Dunlevy, Burren, Chitty, et al., 2006**).

Because SAH accumulation potently inhibits methyltransferase activity and is normally cleared via conversion to homocysteine, a cellular shift favoring SAM over SAH signals reduced capacity for ongoing methylation. Consistent with a functional role for methylation in neurulation, Linden and colleagues observed widening of the anterior neuropore in chick embryos exposed to methylation inhibitors (**Van der Linden et al., 2008**), while Toriyama and colleagues linked methylation disruption to impaired neural tube closure, potentially through effects on ciliogenesis, a cytoskeletal process integral to closure (**Toriyama et al., 2017**).

The full scope of methylation's influence on neurulation and related pathways continues to be defined. The one-carbon transfers central to folate metabolism generate byproducts, including homocysteine, that must be cleared. Steegers-Theunissen and colleagues reported that women with a history of NTD-affected pregnancies exhibited elevated plasma homocysteine despite normal folate levels, raising the possibility that homocysteine may serve as a marker of underlying folate metabolic dysfunction (**Brouwer et al., 2000**). The C677T variant of the MTHFR gene, a well-established cause of elevated plasma homocysteine, has been observed more frequently among mothers of NTD-affected offspring than among controls; nevertheless, certain murine models of hyperhomocysteinemia have not shown correspondingly elevated NTD incidence (**Bennett et al., 2006; Greene, Dunlevy, & Copp, 2003**). Seeking to reconcile these findings, Yang and colleagues conducted a systematic review and meta-analysis identifying a modest elevation in maternal plasma homocysteine among mothers of NTD-affected offspring, while cautioning that heterogeneous blood sampling timing and inconsistent folate supplementation status across studies complicate interpretation (Yang **et al., 2016**). The precise contribution of homocysteine metabolism to NTD risk therefore remains unresolved and merits continued investigation.

The substantial decline in NTD incidence observed today is attributable in large part to the prevention of folate deficiency, a risk factor first proposed in the 1960s but not widely recognized until the late 1980s and early 1990s (**Schorah, 2009**). A body of observational and randomized evidence linking maternal folate status to NTD incidence ultimately established the current recommendation of 4 mg/day for high-risk women, defined as those with a prior NTD-affected pregnancy (**Crider, Bailey, & Berry, 2011**). Strategies for increasing folate intake have included dietary modification, supplementation, and food fortification; in the United States, the FDA authorized folate fortification in 1996 and mandated it by 1998, and as of 2021, more than 78 countries had implemented mandatory folic acid fortification of flour. Nonetheless, many countries have yet to adopt fortification policies and continue to bear a substantial burden of NTD-affected births (**Rosenthal et al., 2014**). Folate deficiency is clearly established as a cause of NTDs, yet both murine models and human populations exist in which NTDs occur despite adequate folate supplementation a reminder of the intricate interplay between genetic and environmental factors that continues to define this condition.

Pathophysiology

As established above, the underlying insult in SB stems from a primary failure of neurulation, which leaves the developing spinal cord directly exposed to the fetal environment rather than shielded as it would normally be. The dominant explanatory framework for how this exposure culminates in permanent neurological injury is the "two-hit" hypothesis, first proposed by Heffez and colleagues based on experimental work in fetal rat and porcine models.

Under this model, the exposed neuroepithelium initially differentiates and develops in a largely normal fashion; however, subsequent "second-hit" insults, including direct exposure to amniotic fluid and mechanical trauma sustained by the unprotected cord in utero, progressively drive neuronal injury and cell loss. This framework has since been reinforced by work from Stiefel and colleagues in mutant mouse models (**Stiefel, Copp, & Meuli, 2007**), as well as by clinical observations of infants who display an abrupt postnatal decline in motor function despite apparently normal movement patterns detected in utero.

This mechanistic understanding of neurological injury in NTDs provided the impetus for pursuing fetal, rather than exclusively postnatal, defect closure, on the premise that earlier intervention could limit cumulative mechanical and amniotic-fluid-related damage to the exposed cord. Further support for this approach came from a fetal lamb model demonstrating that hindbrain herniation characteristic of Chiari malformation could be reversed by in utero closure of the open spinal defect evidence suggesting that the functional deficits associated with NTDs might themselves be recoverable through prenatal repair (**Paek et al., 2000**).

No single medical or surgical intervention can fully reverse the constellation of deficits associated with SB and MMC; nonetheless, a range of interventions meaningfully improves affected patients' quality of life. Initial management centers on basic stabilization and resuscitation maintaining thermoregulation, supplemental oxygen, and airway clearance as needed while taking care to protect the exposed spinal cord from further injury in cases not repaired prenatally (**Wyckoff et al., 2015; Perlman et al., 2015**). Vigilance for cranial nerve compromise and airway protection is critical in the immediate newborn period, as airway compromise secondary to swallowing dysfunction in infants with severe Chiari II malformation represents the leading cause of neonatal death in this population.

Clinical Management and Complications

1. Chiari II malformation and hydrocephalus

Chiari II malformation caudal displacement of the cerebellum and brainstem through the foramen magnum is a near-universal accompaniment of MMC (**Copp et al., 2015**). This malformation is thought to arise from a traction effect exerted on the brainstem by the open MMC defect below, producing characteristic elongation of the medulla, brainstem, and fourth ventricle. The resulting disruption of CSF circulation raises intraventricular pressure and precipitates secondary hydrocephalus, though hydrocephalus can also occur in SB independent of MMC or Chiari II malformation. Because CSF circulation is disrupted, most affected patients ultimately require ventriculoperitoneal shunting to divert excess CSF from the cerebral ventricles into

the abdominal cavity; the likelihood of requiring shunting correlates loosely with lesion level, with more rostral lesions carrying a greater likelihood of needing intervention (**Rintoul et al., 2002**). Shunt-related complications infection and mechanical malfunction chief among them frequently necessitate operative revision or replacement, and malfunction that goes unrecognized or untreated can progress to severe hydrocephalus with associated brain injury.

2. Bowel, bladder, and sexual dysfunction

Bowel and bladder dysfunction, alongside lower extremity paralysis, affect many SB patients to a degree determined by the level of spinal cord involvement. Management of urinary function draws on multiple complementary strategies, spanning pharmacologic approaches to improve continence, surgical continence procedures, and clean intermittent catheterization for effective bladder emptying (**Mariani et al., 2025**). Inadequately managed bladder dysfunction risks progression to hydronephrosis and, ultimately, renal impairment necessitating dialysis or transplantation. Bowel management likewise demands sustained, dedicated attention from infancy through childhood, ranging from first-line laxatives, enemas, and suppositories to surgical approaches including, in some cases, colostomy when conservative measures prove insufficient.

As survival into adulthood has become increasingly common among individuals with SB, attention has correspondingly turned toward adult-specific health concerns, including sexual function, an area in which data remain comparatively sparse. Two systematic reviews, by Streur and colleagues and by Hughes and colleagues, each identified a consistent pattern of sexual dysfunction affecting both men and women with SB (**Streur et al., 2021; Hughes et al., 2021**). Among men, though a substantial proportion (56–96%) retain the capacity for erection, the erection achieved is often inadequate for penetration, and ejaculation, when it occurs, is frequently dribbling or retrograde, evidenced by semen within the urine; only 20–67% of men report the ability to reach orgasm. Among women, sexual desire appears relatively preserved, though dyspareunia occurs across a meaningful proportion of patients, likely secondary to pelvic organ prolapse, and orgasmic capacity is similarly diminished (**Choi, Kim, Ji, et al., 2018**). These findings warrant cautious interpretation given the small sample sizes and reliance on questionnaires not formally validated in this population that characterize much of this literature; the reviews' authors emphasize a corresponding need to initiate sexual health conversations earlier in adolescence to help normalize sexual activity and well-being.

3. Extremity motor function

Motor impairment is a well-established feature of SB, manifesting across a spectrum from isolated motor and sensory deficits to significant difficulty with standing and walking. The degree of motor impairment tracks closely with lesion level, with ambulatory capacity diminishing as lesions occur more proximally (**Showalter et al., 2024**). Deficits tend to become more pronounced as children grow older and heavier. Survival to adulthood is uncommon among patients with cervical- or thoracic-level lesions, and those with lesions at L2 or above are almost universally

wheelchair-dependent; by contrast, patients with lower lumbar (L5) or sacral-level lesions typically retain greater motor strength and more often ambulate into adulthood (**Sival et al., 2004; Dicianno et al., 2015**).

Overall, roughly three-quarters of individuals born with spina bifida survive into adulthood. Orthopedic foot deformities, such as clubfoot, are also commonly associated with SB and warrant early referral to orthopedic specialists (**May, DeMaio, & Larson, 2025**). Given this complexity, comprehensive SB care necessarily involves a multidisciplinary team spanning multiple pediatric subspecialties physiatry, general surgery, neurosurgery, urology, orthopedics, and gastroenterology supported by physical therapists, social workers, and care coordinators. The central role families play in navigating the physical and social dimensions of this condition merits equal emphasis (**Mannino et al., 2024**).

4. Fetal intervention

For roughly a century, standard management of SB consisted of surgical closure of the open defect within 48 hours of birth, primarily to reduce infection risk; this postnatal approach, however, cannot reverse spinal cord damage already sustained from secondary in utero injury. Recognition of this limitation motivated early attempts at fetal repair, culminating in the randomized Management of Myelomeningocele Study (MOMS), designed to compare intrauterine repair performed between 19 and 25 weeks' gestation against standard postnatal repair (**Adzick et al., 2011**).

The trial's Data Safety and Monitoring Board halted enrollment in December 2010 after 183 of a planned 200 participants on the basis of demonstrated efficacy favoring fetal surgery. Specifically, MOMS demonstrated a marked reduction in the need for ventriculoperitoneal shunt placement by one year of age among infants who underwent fetal repair (40%) compared with postnatal repair (82%), alongside broader neuromotor gains: 42% of children in the fetal surgery group achieved independent ambulation without orthotics or devices, versus 21% in the postnatal group, findings subsequently corroborated at 30-month follow-up (**Adzick et al., 2011; Farmer et al., 2018**). Prenatally repaired infants also achieved higher composite scores on combined measures of mental and motor development, alongside improvements in secondary outcomes including hindbrain herniation and likelihood of independent ambulation.

A secondary analysis of 6-year MOMS outcomes by Houtrow and colleagues extended these findings, showing that prenatally repaired children demonstrated superior gait quality, achieved higher-level mobility skills, and were less likely to exhibit motor function below what would be predicted by their anatomic lesion level (**Houtrow et al., 2021**).

Sustained long-term follow-up of the MOMS cohort remains essential, both to confirm durability of these benefits and to characterize their downstream impact on quality of life for patients and families. Families facing a pregnancy complicated by SB currently face several pathways: pregnancy termination, cesarean delivery followed by postnatal repair, or fetal repair, now considered state-of-the-art for eligible candidates.

Although fetal intervention meaningfully improves Chiari-related outcomes, gains in distal extremity neurologic function, while encouraging, remain incomplete,

with 58% of treated children still unable to achieve independent ambulation. Compounding this, many centers nationally still lack the specialized infrastructure, training, and support staff required to offer fetal intervention, meaning that despite its promise, access remains geographically and institutionally limited. A detailed discussion of the indications, contraindications, and surgical techniques underlying fetal repair falls outside the scope of this review; however, an emerging and closely related area the incorporation of stem cell therapy into fetal treatment warrants dedicated discussion.

5, In Utero Cellular Therapies

Interest in cellular therapy as a means of augmenting prenatal MMC repair has grown substantially over the past two decades. Investigational efforts have progressed through several stem cell platforms human embryonic stem cells, neural crest stem cells, induced pluripotent stem cells, human amniotic fluid-derived stem cells, and, most recently, mesenchymal stem cells largely evaluated across murine, ovine, and avian model systems.

Lee and colleagues were the first to investigate human embryonic stem cells (hESCs) in this context, using a chicken embryo model of MMC in which hESCs were injected into the amniotic cavity of a surgically created open spinal NTD, delivered either immediately or 24 hours following incision (**Lee, Kim, Park, et al., 2006**). Relative to control and vehicle-treated groups, hESC-treated defects achieved significantly greater reclosure; the investigators proposed that this likely reflected secreted reparative factors growth factors and neurotransmitters among them along with a mechanical bridging or "glue-like" effect, rather than direct replacement of lost cells. Building on this, the same group went on to compare a neural stem cell line (F3) against human bone marrow-derived stem cells (B10) in the identical model, finding that bone marrow-derived cells meaningfully enhanced NTD reclosure whereas the neural stem cell line failed to do so a shortfall attributed to poor stem cell survival (**Lee, Phi, Kim, et al., 2010**).

Fauza and colleagues subsequently introduced prenatal neural stem cells (NSCs) as a therapeutic strategy for fetal SB repair, reasoning that NSC-derived neurotrophic and neuroprotective factors could support survival and regeneration of neural tissue; using a fetal lamb model, they demonstrated that transplanted NSCs remained undifferentiated and continued producing neurotrophic factors following delivery to the fetal spinal cord (**Fauza et al., 2008**).

Saadai and colleagues extended this work to induced pluripotent stem cells (iPSCs), delivering human iPSC-derived neural crest stem cells generated from skin fibroblasts into the injured spinal cord in a fetal lamb model of MMC (**Saadai et al., 2013**). These cells demonstrated greater than 95% viability and underwent neuronal differentiation in vitro; in vivo, they survived, integrated into host tissue, and differentiated along a neuronal lineage. Separately, amniotic fluid-derived human iPSCs were applied to artificial skin constructs in a rat model of MMC, where addition of Y-27632 and epidermal growth factor (EGF) drove differentiation into keratinocytes **(Kajiwara et al., 2017).**

More recently, mesenchymal stromal cells (MSCs) sourced from multiple tissue

origins have generated considerable promise. Abe and colleagues first proposed amniotic fluid-derived MSC therapy as a prenatal treatment strategy in a rat model of MMC, with hepatocyte growth factor secretion proposed as the mechanism driving spinal cord coverage (**Abe et al., 2019**). In a rabbit model, Shieh and colleagues subsequently showed that concentrated intra-amniotic MSC injection, termed trans-amniotic stem cell therapy (TRASCET), achieved partial or complete skin-like coverage of the SB defect in both rodents and rabbits relative to controls (**Shieh et al., 2019**). Bone marrow- and placenta-derived mesenchymal stem cells have similarly improved spinal cord reclosure and ambulation in rodent and ovine SB models, an effect linked to expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (**Li et al., 2012; Kabagambe et al., 2017; Wang, Brown, Lankford, et al., 2015**), with subsequent work additionally identifying elevated EGF and fibroblast growth factor (FGF) expression within transplanted cells (**Li, Miao, Zhao, et al., 2014**).

This preclinical program of work spanning murine, ovine, and rabbit models across nearly two decades culminated in the CuRe Trial (Cellular Therapy for In Utero Repair of Myelomeningocele), the first FDA-approved clinical trial of stem cell therapy administered at the time of intrauterine MMC repair. Led by investigators at UC Davis Health, the trial applies allogeneic placenta-derived mesenchymal stem cells seeded on an FDA-approved extracellular matrix directly to the exposed fetal spinal cord during standard open fetal surgery. Phase 1 results, published in *The Lancet* in February 2026, demonstrated feasibility and safety in the first six treated patients: all infants were born with intact repair sites and no cerebrospinal fluid leak, infection, wound dehiscence, or abnormal tissue growth attributable to the cellular therapy, meeting the prespecified safety threshold for the trial to proceed to its next phase (**Farmer, Kumar, Reynolds, et al., 2026**). Longer-term follow-up to 30 months of age, and a planned expansion to 35 total participants, will be needed to determine whether the therapy meaningfully improves motor outcomes beyond what fetal surgery alone achieves.

Additional Complications and Surgical Risks

1. Spina bifida-related complications

Bladder and bowel dysfunction stands out as the most frequently encountered complication in this population, carrying substantial risk for acute renal failure and urosepsis, typically driven by vesicoureteral reflux secondary to an underlying neurogenic bladder. Relative to the general population, affected individuals face a 7- to 11-fold elevation in renal failure risk, roughly double the risk of bladder cancer, and up to a 46-fold increase in urinary tract infections. Given these stakes, vigilant urologic surveillance paired with proactive treatment is essential, and a considerable proportion of patients will eventually require intermittent catheterization to manage bladder emptying. Neurogenic bladder itself is highly common, with prevalence reaching as high as 80%. Video-urodynamic testing is the recommended approach for excluding vesicoureteric reflux, while surgical bladder augmentation becomes indicated once detrusor overactivity no longer responds to medical management (**Williams et al., 2002**).

Tethered spinal cord and hydromyelia represent additional recognized

complications, which may present as sudden neurological deterioration, worsening spasticity, or new-onset pain. Individuals with neural tube defects also face heightened fracture risk, attributable to osteopenia, joint contractures, diminished sensation, and reduced mobility. When retethering recurs, vertebral column resection may become a necessary surgical option (**Yamaguchi et al., 2011**).

Scoliosis is another commonly seen complication, affecting roughly 33% of patients, followed by chronic pain in about 29% and epilepsy in approximately 12%. Other documented complications associated with spina bifida include:

- Motor impairment
- Hydrocephalus and Arnold–Chiari type II malformation
- Clubfoot (present in nearly 50% of cases)
- Latex sensitivity (reported prevalence between 10% and 73%)
- Working memory deficits, with academic difficulty most notable in English, mathematics, and history
- Increased susceptibility to eating disorders and body image concerns
- Broader psychosocial effects
- Difficulty transitioning from pediatric to adult healthcare systems
- Ongoing need for lifelong self-management
- Caregiver strain, including adverse experiences and practical challenges for those providing care (**Yazdy et al., 2011; Hensel, Young, & Szymanski, 2025**)

2. Surgical complications

Surgical management of spina bifida carries its own set of potential risks:

- Infection, with elevated risk when surgery is delayed or antibiotic prophylaxis is withheld
- Retethering, occurring in roughly 20% of cases
- Pseudomeningocele formation
- Pressure-related tissue injury during prolonged procedures, preventable through intraoperative pressure mapping
- Cerebrospinal fluid (CSF) fistula (**Yun et al., 2024; Etter et al., 2025)**

CONCLUSION

Spina bifida remains the most common congenital malformation of the central nervous system and a significant source of lifelong morbidity, yet the past several decades have transformed our understanding of both its origins and its treatment. What was once viewed primarily as a folate-deficiency disorder is now recognized as a genuinely complex trait, shaped by the interplay of dozens of implicated genes spanning planar cell polarity signaling, Wnt/β-catenin regulation, and folate-homocysteine metabolism, alongside maternal metabolic status, medication exposure, and other environmental influences. The emerging omnigenic model, increasingly supported by whole-exome sequencing data, suggests that no single genetic or environmental factor will fully explain an individual patient's risk, and that meaningful progress toward prevention and personalized counseling will depend on continued, large-scale genomic characterization of affected populations.

Parallel advances in understanding disease mechanism have proven equally

consequential. The recognition that neurological injury in SB reflects not a single developmental insult but a "two-hit" process — an initial failure of neurulation compounded by ongoing mechanical and chemical trauma in utero reframed the therapeutic question from one of static defect closure to one of active, time-sensitive neuroprotection. This shift in thinking directly enabled the transition from postnatal to prenatal surgical repair, culminating in the landmark MOMS trial and its durable improvements in shunt-independence and motor outcomes. Yet even optimal fetal surgery leaves a majority of children with persistent motor deficits, underscoring that mechanical closure alone cannot fully arrest the injury cascade.

It is this gap that cellular therapy is positioned to address. Decades of preclinical work across embryonic, neural, induced pluripotent, and mesenchymal stem cell platforms have converged on the current generation of placenta-derived mesenchymal stromal cell therapies, now being tested in the CuRe Trial the first FDA-approved clinical trial of its kind. Phase 1 results, published in early 2026, establish that adding this cellular therapy to standard fetal surgery is feasible and safe; whether it further preserves neurologic function beyond what surgery alone achieves is the question later phases of the trial are designed to answer. Should this and future trials confirm a functional benefit, the field will have moved meaningfully closer to a therapy that addresses the underlying injury of spina bifida rather than only its anatomic consequence, offering renewed hope to the patients and families who live with this disease.

Author Contributions

Hasibullah Ayami conceptualized the review, developed the study design, supervised the project, interpreted the scientific literature, drafted major sections of the manuscript, and critically revised the final version. Aliseena Yussufpur performed the comprehensive literature search, screened and organized the relevant publications, contributed to data interpretation, prepared several sections of the manuscript, participated in critical scientific discussions, and assisted in editing and formatting. Abdul Hasib Azarakhsh corresponding the paper and cooperate in case of submitting the paper to concerning journal.

All authors contributed substantially to the intellectual content of the manuscript, approved the final version for publication, and agree to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare that there are no financial, personal, institutional, professional, or commercial relationships that could be construed as a potential conflict of interest regarding the publication of this manuscript. The authors have no competing interests related to the subject matter discussed in this review. Furthermore, no funding body or external organization influenced the design, interpretation, writing, or publication of this work.

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