Spina bifida

Andrew J. Copp[1], N. Scott Adzick[2], Lyn S. Chitty[3], Jack M. Fletcher[4], Grayson N. Holmbeck[5] and Gary M. Shaw[6]

Abstract | Spina bifida is a birth defect in which the vertebral column is open, often with spinal cord involvement. The most clinically significant subtype is myelomeningocele (open spina bifida), which is a condition characterized by failure of the lumbosacral spinal neural tube to close during embryonic development. The exposed neural tissue degenerates *in utero*, resulting in neurological deficit that varies with the level of the lesion. Occurring in approximately 1 per 1,000 births worldwide, myelomeningocele is one of the most common congenital malformations, but its cause is largely unknown. The genetic component is estimated at 60–70%, but few causative genes have been identified to date, despite much information from mouse models. Non-genetic maternal risk factors include reduced folate intake, anticonvulsant therapy, diabetes mellitus and obesity. Primary prevention by periconceptional supplementation with folic acid has been demonstrated in clinical trials, leading to food fortification programmes in many countries. Prenatal diagnosis is achieved by ultrasonography, enabling women to seek termination of pregnancy. Individuals who survive to birth have their lesions closed surgically, with subsequent management of associated defects, including the Chiari II brain malformation, hydrocephalus, and urological and orthopaedic sequelae. Fetal surgical repair of myelomeningocele has been associated with improved early neurological outcome compared with postnatal operation. Myelomeningocele affects quality of life during childhood, adolescence and adulthood, posing a challenge for individuals, families and society as a whole. For an illustrated summary of this Primer, visit: http://go.nature.com/fK9XNa

Spina bifida is a congenital malformation in which the spinal column is split (bifid) as a result of failed closure or formation of the embryonic neural tube. The most common and severe form is myelomeningocele (also termed open spina bifida or spina bifida aperta) (FIG. 1), which is the focus of this Primer. In myelomeningocele, the spinal cord is open dorsally, forming a placode on the back of the fetus or newborn baby; this placode frequently rests on a meningeal sac (then termed spina bifida cystica[1]). The vertebrae at the level of the lesion lack neural arches and are incomplete dorsally.

Individuals with myelomeningocele often exhibit neurological deficits below the level of the lesion, involving both motor and sensory functions. This deficit might result in lower-limb weakness or paralysis that hinders or prevents walking, and lack of sensation that enhances the risk of pressure sores. Urinary and faecal incontinence occurs frequently, as do hindbrain herniation (the Chiari II malformation) and associated hydrocephalus, which often requires shunting. Orthopaedic abnormalities such as talipes (club foot), contractures, hip dislocation, scoliosis and kyphosis are frequently observed. A strong correlation is apparent between the axial level of lesion and the degree of disability experienced by patients. A 40-year follow-up study of 117 children whose lesions were repaired in the United Kingdom during the 1960s and 1970s found a 17% survival rate among those with lesions above the eleventh thoracic vertebra (T11); by contrast, 61% of individuals with lesions below the third lumbar vertebra (L3) were alive at the end of the study[2]. Fewer survivors were mobile (community walkers) and free of pressure sores in the 'above T11' group than in the 'below L3' group.

The lifetime cost of caring for a child born with myelomeningocele is estimated at more than €500,000 (US$600,000), comprising €185,000 ($222,000) in direct medical costs and the remainder in indirect costs, including special educational and care-giver needs, and loss of employment potential[3]. In view of these life-changing health and economic consequences, considerable effort has been invested in exploring the pathophysiological mechanisms of the disorder, finding better ways to treat and manage the condition and its consequences, and progressing towards the ultimate goal of primary prevention. This Primer considers the main areas of progress to date and discusses developments that might further improve the outlook for individuals with myelomeningocele.

Correspondence to A.J.C.
e-mail: a.copp@ucl.ac.uk
Newlife Birth Defects Research Centre, University College London Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

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Author addresses

[1]Newlife Birth Defects Research Centre, University College London Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.
[2]Department of Surgery, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA.
[3]Genetics and Genomic Medicine, University College London (UCL) Institute of Child Health, Great Ormond Street Hospital for Children NHS Foundation Trust and UCL Hospitals NHS Foundation Trust, London, UK.
[4]Department of Psychology, University of Houston, Houston, Texas, USA.
[5]Department of Psychology, Loyola University Chicago, Chicago, Illinois, USA.
[6]Department of Pediatrics, Stanford University School of Medicine, Stanford, California, USA.

Epidemiology

Many epidemiological studies group spina bifida together with the related defect anencephaly, and sometimes also with encephalocele, under the general term neural tube defects (NTDs) (FIG. 1). The prevalence of NTDs has varied considerably over the past four decades[4] and continues to show substantial differences between geographical locations. For example, the prevalence of NTDs in the United States and many European countries is estimated at 0.5–0.8 cases per 1,000 births[5], whereas the prevalence in some regions of China has been reported to be more than 20 times as high[6]. Assuming an average prevalence of one NTD case per 1,000 births, with a global population of 7 billion and a yearly birth rate of 20 per 1,000 individuals, this generates a figure of 140,000 NTD cases per year worldwide. Regions of higher NTD prevalence have uniquely shown disproportionately high frequencies of rare subtypes such as craniorachischisis and iniencephaly[7]. Further, within-country differences have been observed between racial and ethnic groups. For example, in the United States, the prevalence of spina bifida is higher in Hispanics[8] and lower in African Americans[9] than in non-Hispanic whites. Prevalence differences in time and across geographical regions have been attributed to variations in ascertainment methods, as well as to true differences in risk. Indeed, the ascertainment of NTD prevalence is challenging, as antenatal screening procedures can lead to diagnosis and subsequent pregnancy termination. Accordingly, the omission of such elective terminations from the data clearly leads to an underestimation of NTD prevalence and might bias risk estimations in aetiological studies[10]. EUROCAT, the European network of population-based registries for epidemiological surveillance of congenital anomalies, collects data on pregnancy terminations in addition to live births and stillbirths, generating particularly comprehensive prevalence data for NTDs and other malformations. For the period 2003–2007, EUROCAT estimated the prevalence of spina bifida and NTDs (including chromosomally related disorders) at 0.51 and 0.94, respectively, per 1,000 births, stillbirths and pregnancy terminations[11].

Both genetic and non-genetic factors contribute to NTDs. Heritability (the genetic component of risk) was estimated at 60–70% on the basis of the relative proportions of affected siblings as analysed in prevalence surveys in the 1960s in South Wales, Glasgow and London, UK[12]. Fewer than 10% of NTDs are syndromic — for example, occurring in chromosomal disorders such as trisomy 13 or trisomy 18 — whereas the great majority are non-syndromic and exhibit a sporadic pattern of occurrence. Several lines of evidence support a multifactorial causation model for non-syndromic NTDs, involving multiple genes and non-genetic factors[13]. The recurrence risk for siblings of an index case is 2–5%, representing a 20-fold to 50-fold increase in risk compared with the general population[4]. Second-degree and third-degree relatives show lower recurrence risks than first-degree relatives, but still higher risks than unrelated individuals. For a particular woman, the empirical recurrence risk for subsequent offspring with NTDs after an affected pregnancy is ~3%, rising to ~10% after two affected pregnancies[4]. In twins, the concordance for NTDs is higher among same-sex twin pairs (both monozygotic and dizygotic) than among opposite-sex twin pairs. The finding that more fetuses and infants with anencephaly are female, has strongly suggested a sex-related genetic or epigenetic component to anencephaly[14]. Finally, the prevalence differences between ethnic groups have been reported to persist in some cases after migration to other geographical locations[15]. Hence, considerable evidence points to a major genetic component in spina bifida causation, raising the question of which genes are implicated.

Considering non-genetic factors, diminished folate status is undoubtedly the best-known factor influencing NTD risk. Beyond folate, a number of other nutrients and nutrition-related factors have been linked with NTDs (BOX 1). The association with maternal obesity is particularly notable and has been consistently reported in studies from a variety of populations worldwide[16]. Interestingly, these maternal obesity-associated risks are stronger for spina bifida than for anencephaly[16–18] and might not be reduced by maternal folic acid use[19]. For spina bifida, elevated risks in the range of 1.5-fold to 3-fold have been consistently observed. In addition, severe maternal obesity (a body mass index of >35) has been associated with even larger risks for the offspring, indicative of a dose–response relationship linking maternal obesity with spina bifida. Suggested mechanisms underlying this potential link include aberrant glucose control, oxidative stress and metabolic syndrome[18]. Other non-genetic factors that have been linked with NTDs include exposure to a variety of environmental factors, such as pollutants and personal toxicants (BOX 1). However, most of these factors have not been consistently observed, are relatively infrequent in occurrence, or have not shown a high magnitude of risk. Thus, such factors are unlikely to explain a substantive proportion of the population burden of NTDs[20].

Mechanisms/pathophysiology

The primary disorder in the pathogenesis of myelomeningocele is failed neural tube closure in the embryonic spinal region, which leads to prolonged exposure of the open neural tube to the amniotic-fluid environment. Remarkably, the bifid neuroepithelium initially undergoes relatively normal neuronal differentiation, with the development of spinal motor and sensory function even below the lesion level. As gestation

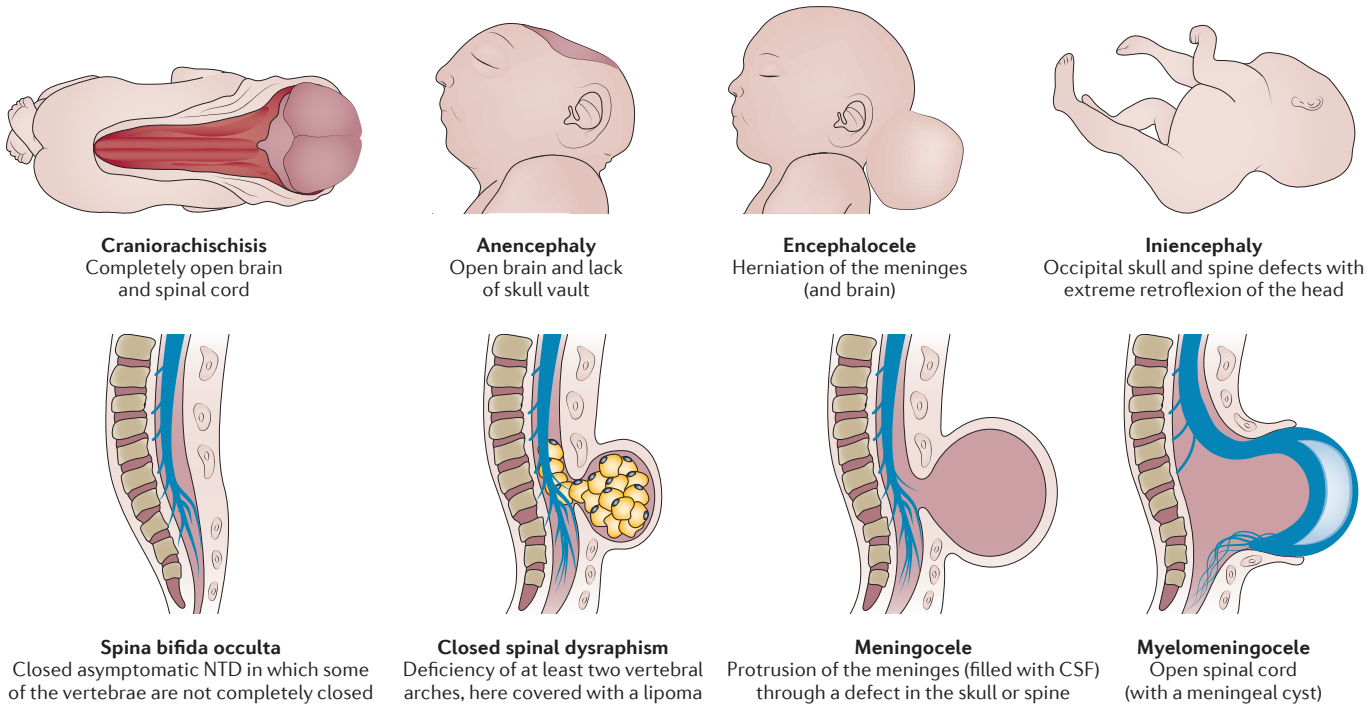

Figure 1 | **Overview of neural tube defects.** Schematic representation of several neural tube defects (NTDs). Spina bifida occulta is found in up to 10% of people and usually occurs in the low spinal region. Closed spinal dysraphism has many variants, including lipomyelomeningocele, low-lying conus and thickened filum terminale. CSF, cerebrospinal fluid.

Box 1 | **Potential risk factors for neural tube defects**

Maternal nutrition
- Alcohol use[198]
- Caffeine use[199]
- Low folate intake[200]
- Low dietary quality[201]
- Elevated glycaemic load or index[202]
- Low methionine intake[203]
- Low serum choline level[204]
- Low serum vitamin B12 level[205]
- Low vitamin C level[206]
- Low zinc intake[207]

Other maternal factors
- Smoking[198]
- Hyperthermia[208]
- Low socio-economic status[209]
- Maternal infections and illnesses[210]
- Pregestational insulin-dependent diabetes[211]
- Pregestational obesity[17]
- Psychosocial stress[212,213]
- Valproic acid use[214]

Environmental factors
- Ambient air pollution[215,216]
- Disinfectant by-products in drinking water[217]
- Indoor air pollution[218]
- Nitrate-related compounds[219]
- Organic solvents[220]
- Pesticides[221,222]
- Polycyclic aromatic hydrocarbons[223]

progresses, however, the exposed spinal cord becomes haemorrhagic, and neurons die as a result of the toxicity of the amniotic fluid (BOX 2). Axonal connections are interrupted and function is lost[21]. Hence, the neurological disability is often considered a two-hit process: failed neural tube closure followed by neurodegeneration *in utero*. Accordingly, attempts to cover the lesion during fetal development have been encouraged, to arrest or prevent neurodegeneration in individuals for whom closure has failed.

Genetic factors

More than 200 genes are required for successful neural tube closure in mice, with new examples of essential genes being described on a regular basis[22]. These genes relate to a wide range of cellular functions (such as cytoskeletal regulation, transcriptional regulation and cell proliferation control) and molecular pathways (including Hedgehog, bone morphogenetic protein and retinoid signalling)[23], and mutant models display a variety of phenotypes that mimic the range of human NTDs. Exencephaly, the developmental precursor of anencephaly, is the most commonly encountered NTD following mutation of these genes in mice and is associated with more than 150 genes. However, myelomeningocele is observed in more than 40 mutant mouse strains, often in combination with exencephaly but in several cases as the only NTD[22,24]. For many of these genes, sequencing of the coding regions of human orthologues has revealed that patients with NTDs often carry rare missense (that is, amino acid-altering) mutations that are absent from unaffected

Box 2 | **Evidence for progressive injury of the exposed spinal cord *in utero***

- Pathological examination of the spinal cords of stillborn human fetuses with myelomeningocele demonstrate varying degrees of neural tissue loss at the site of the defect, but normal-appearing dorsal and ventral horns proximal of the lesion[1,224].
- Serial sonographic observations of human fetuses with myelomeningocele show progressive deterioration of leg movements during gestation[225,226].
- In hemimyelocele, half of the dysraphic spinal cord is devoid of dura and openly exposed to the intrauterine environment; the corresponding lower extremity shows impaired function, whereas function is normal or only mildly diminished in the extremity connected to the covered part of the spinal cord[227].
- In animal models, staged series of fetuses with myelomeningocele have demonstrated gain of neurological function even after the lesion has formed, followed by loss of this function. This finding correlates with a progressive loss of spinal cord tissue integrity[21,228].
- Human amniotic fluid develops a sudden toxicity at 34-weeks gestation, as judged by cell death in organotypic cultures of rat spinal cord[229].

individuals. In particular, variants of genes involved in the planar cell polarity pathway (a non-canonical WNT signalling cascade) have been associated with a range of NTDs[25]. This finding is particularly important because gene mutations in this pathway often result in NTDs in mice, generating several phenotypes, including the severe malformation craniorachischisis, which is an almost completely open brain and spine[26]. A second group of NTD-associated genes are those encoding enzymes that function in folate one-carbon metabolism, which is essential both for the synthesis of nucleotides (as the building blocks of DNA) and for methylation reactions that control gene expression and other cellular functions. Included in this enzyme group is 5,10-methylenetetrahydrofolate reductase (encoded by *MTHFR*), an enzyme that produces 5-methyltetrahydrofolate, which is essential for the conversion of homocysteine into methionine. The *MTHFR* C677T variant, which results in the conversion of valine to alanine at codon 222, reduces the activity of this enzyme. Indeed, this mutation in either the mother or fetus can be a risk factor for NTDs in populations of non-Latin origin, particularly when the folate status of the mother is low[27]. However, in mice, *Mthfr* knock out does not generate NTDs[28], raising a question about the specificity of this genetic association with NTDs. By contrast, mutations in genes of the glycine cleavage system have been found among patients with NTDs and, in this case, loss of function of the mouse orthologues also produces NTDs[29,30]. These mutations in the glycine cleavage system genes reduce the activity of glycine decarboxylase and amino methyl transferase, which mediate the breakdown of glycine within mitochondria, a key step in cellular folate one-carbon metabolism.

Non-genetic factors

Although various environmental factors have been linked with NTDs (BOX 1), only a few clues exist about the pathogenetic mechanisms that cause these disorders. Moreover, it seems likely that non-genetic factors influence neural tube closure mainly when combined with a predisposing genotype. The anticonvulsant valproic acid increases risk in the fetus approximately tenfold when taken by the mother during the first trimester of pregnancy[31]. The potent histone deacetylase inhibitory activity of this anticonvulsant might disturb the balance of protein acetylation and deacetylation, leading to failure of neurulation (the folding of the neural plate to create the neural tube)[32]. The fungal product fumonisin was demonstrated to cause NTDs in studies of an 'outbreak' of NTDs in southern Texas, USA, linked to contamination of tortilla flour[33]. Exposure of rodent embryos to fumonisin led to the identification of sphingosine phosphate metabolism as a key target of the toxin; perturbation of this pathway would potentially compromise folate utilization[34]. Maternal diabetes mellitus predisposes the fetus to a range of birth defects, and NTDs occurring in this context are caused by hyperglycaemia, although the pathogenetic mechanism is poorly understood. One suggestion implicates disrupted fetal expression of *PAX3* (REF. 35), loss of function of which leads to NTDs in mice.

Embryonic pathogenesis of myelomeningocele

Two distinct phases of neural tube formation occur in higher vertebrates: primary (closure) and secondary (canalization) (FIG. 2). In humans, primary neurulation is initiated at the boundary between the future hindbrain and the cervical spine on day 22 post-fertilization. From this level, the neural tube 'zips up' bidirectionally up into the hindbrain and down the spine. Closure initiates separately at the rostral extremity of the forebrain, and zipping proceeds backwards from this site to meet the wave of forward closure from the hindbrain. Cranial closure is completed at the rostral neuropore on day 24, whereas spinal closure continues for a longer period, forming progressively lower levels of the neuraxis until it finishes at the caudal (posterior) neuropore on day 26 (REF. 36). This event marks the completion of the spinal cord to the upper sacral level.

NTDs can result from failure of any part of this neurulation sequence and they are typically open defects, owing to the arrest of closure before fusion of the neural folds in the dorsal midline (FIG. 2a–c). The most severe spinal defect is craniorachischisis, in which closure fails to be initiated on day 22. Analyses of mice with mutations in genes involved in the planar cell polarity pathway, including *Vangl2*, have revealed that late gastrulation (the embryonic process resulting in a trilaminar structure comprising ectoderm, mesoderm and endoderm) is defective in these mutant fetuses[37]. The process of convergent extension involves the intercalation of cells in the midline to lengthen and narrow the body axis. When this process fails, as it does in these mouse mutants, the body axis remains short and wide, and the neural folds are spaced abnormally widely apart and are physically unable to initiate closure[37]. If the embryo successfully initiates closure but subsequently fails cranial neurulation, then anencephaly results. By contrast, failure of spinal neurulation following initiation of closure generates open spina bifida lesions of varying sizes and axial levels,

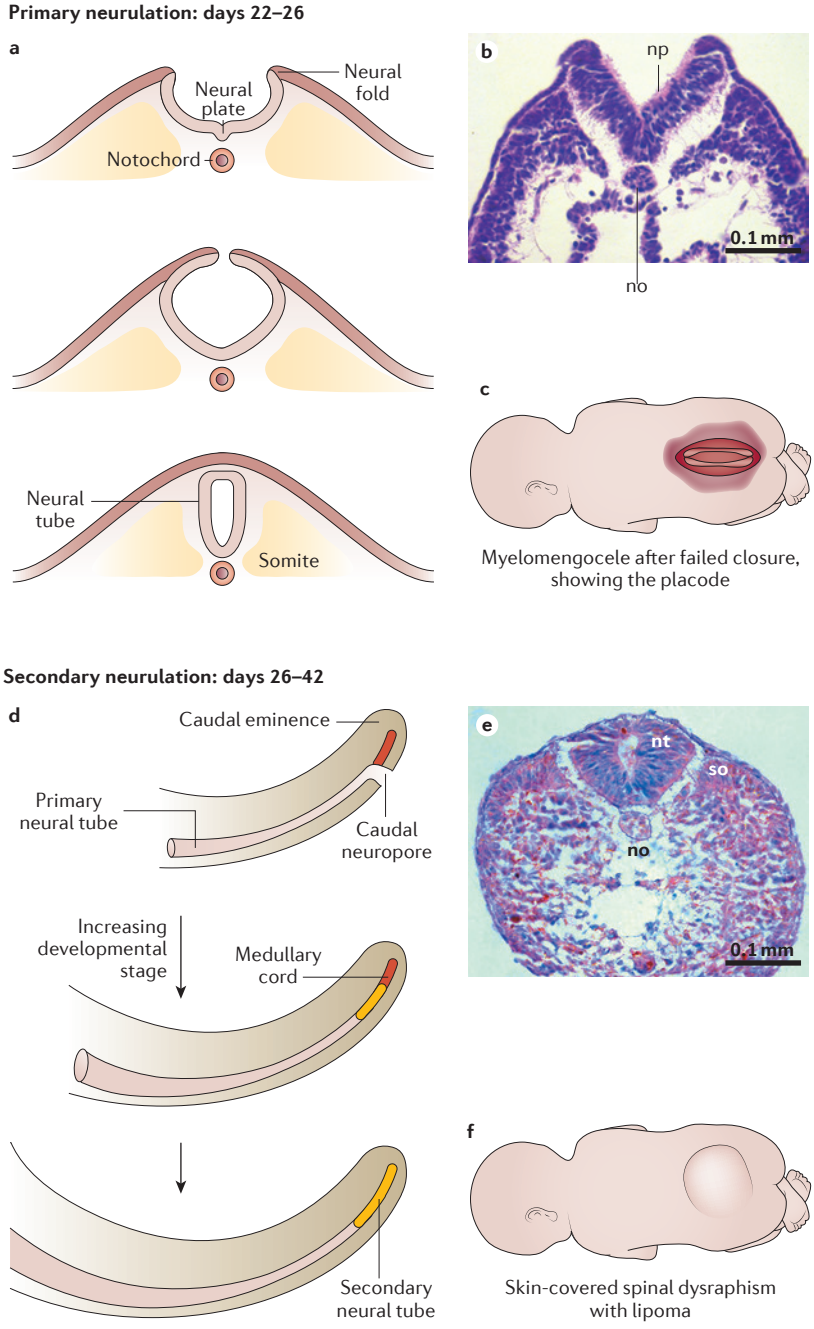

Figure 2 | **Neurulation and the origin of open and closed spinal bifida.** **a** | Schematic transverse sections showing the process of primary neurulation, which involves bending of the neural plate, convergence of the neural folds and closure of the neural tube. **b** | A histological section through the open spinal neural folds of an unaffected human embryo (Carnegie stage 12, 26 days post-fertilization), showing the closing neural tube during primary neurulation. **c** | Failure of the neural groove to close in the low spinal region in the fourth week after fertilization leads to myelomeningocele (also termed open spina bifida). **d** | Schematic sagittal sections showing the process of secondary neurulation, which involves condensation of the caudal eminence, followed by the formation of the lumen (canalization), completion of secondary neurulation and regression of the tail. This process finalizes in the sixth week after fertilization. **e** | A histological section through an unaffected human embryo (Carnegie stage 13, 30 days post-fertilization), showing formation of the secondary neural tube (nt) through canalization. **f** | Failure of the secondary neural tube to separate from non-neural tissues (tethering) leads to closed spinal dysraphism, in this case with massive lipoma. no, notochord; np, neural plate; so, somite.

depending on the stage at which the wave of zipping arrests. For example, *Zic2*-mutant mice fail early in spinal neurulation owing to a lack of dorsolateral neural plate bending[38], and they display a large spina bifida from the thoracic level downwards. By contrast, spinal closure in the curly tail (*Grhl3*-mutant) mouse fails later owing to enhanced curvature of the body axis[39], producing a spina bifida confined to the lumbosacral region. Whether human spina bifida lesions of differing axial extents also result from distinct genetic causes remains unclear.

Pathogenesis of closed spinal dysraphism

Secondary neurulation forms the neural tube in the low sacral and coccygeal regions, following closure of the caudal neuropore (FIG. 2d). The end of the embryo comprises the tail bud (also called the caudal eminence), the mesenchymal cell core of which progressively reorganizes into longitudinal cell condensations. The most dorsal of these condensations undergoes canalization, converting the solid neural precursor into a hollow epithelial secondary neural tube[36,40]. Secondary neurulation does not have a closure component, so defects (closed spinal dysraphism) are not exposed to the external environment, but are skin covered (FIG. 2e,f). The principal defect seems to be failure of the neural and mesodermal tissues to become distinctly specified and spatially separated. The presence of neuromesodermal precursor cells with a bidirectional differentiation potential in the tail bud[41] might explain why this separation is sometimes incomplete. The clinical observation that the distal spinal cord is often tethered to surrounding tissues in closed spinal dysraphism suggests that this form of NTD can be classified as a disorder of secondary neurulation. However, the frequent and striking association of closed spinal dysraphism with intradural lipoma[42] (FIG. 2f) is not well explained and is yet to be reproduced in an animal model.

Postnatal pathogenesis

Among the different forms of spina bifida, myelomeningocele is associated with brain malformations and hydrocephalus. The main brain defects involve the range of anomalies related to the Chiari II malformation of the hindbrain, which is detected in ~90% of cases[43]. This malformation is associated with development of the cerebellum in a small posterior fossa, such that the cerebellum herniates downwards through the foramen magnum[44]. Quantitative studies of these defects show a reorganization of the cerebellum, such that the anterior part is enlarged, the posterior–inferior regions are reduced, and there is no difference in the corpus medullare (cerebellar white matter) compared with a typical cerebellum[45]. Cerebellar volume reduction is more pronounced in thoracic-level spinal lesions than in lumbar or sacral lesions, but volume reductions relative to typically developing controls are observed in all circumstances[46]. In addition, ~65% of patients with myelomeningocele exhibit distortion of the midbrain, often marked by tectal beaking, in which the colliculi fuse into a single beak that points posteriorly and

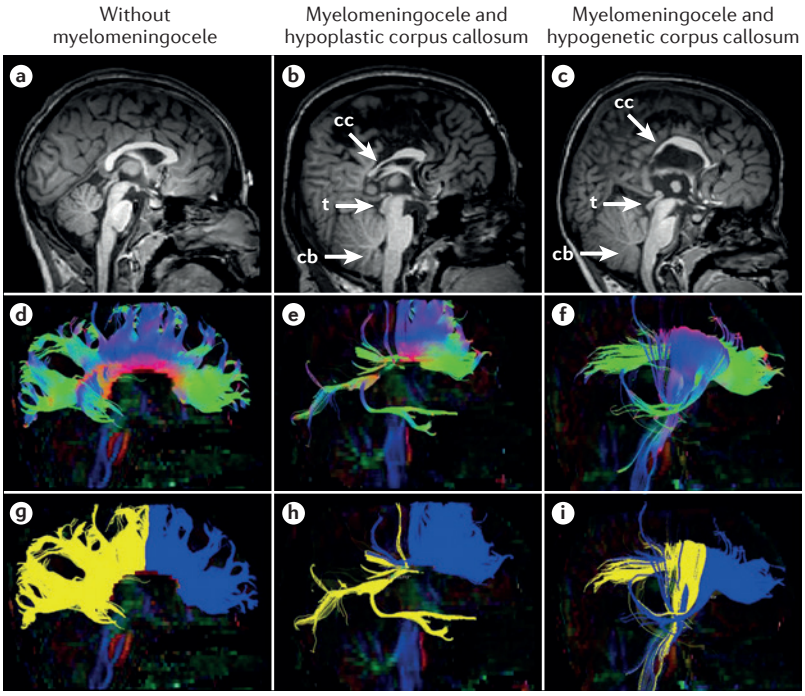

Figure 3 | **MRI appearance of brain dysmorphology in myelomeningocele.** Mid-sagittal MRI images of a typically developing child (parts **a**, **d** and **g**), a child with myelomeningocele and a hypoplastic corpus callosum (parts **b**, **e** and **h**) and a child with myelomeningocele and a hypogenetic corpus callosum (parts **c**, **f** and **i**). T1-weighted MRI images (parts **a**–**c**) that reveal a downward shift of the cerebellum (cb) in the children with spina bifida, representing the Chiari II malformation. Also note the tectal beaking (t) and the structural abnormalities in the corpus callosum (cc). Diffusion imaging tractography (parts **d**–**i**) showing connectivity emanating from the corpus callosum. This connectivity is divided into anterior (frontal; blue) and posterior (yellow) segments (parts **g**–**i**). Note the relative preservation of frontal connectivity in the individuals with spina bifida. There is a greater and more aberrant pattern of connectivity in the child with the hypogenetic corpus callosum. Images courtesy of K. Bradley (University of Houston, Texas, USA) and J. Juranek (University of Texas Health Science Center at Houston, USA).

invaginates into the cerebellum. The medulla is elongated and kinked at the spinomedullary junction in ~70% of patients[43] (FIG. 3a–c).

The basal ganglia and related subcortical structures of individuals with spina bifida are normal on radiological examination[47]. Quantitative macrostructural assessment shows that the hippocampus, but not the amygdala, is reduced in volume[48], and the putamen is enlarged. Approximately one-third to half of children with myelomeningocele have hypogenesis (underdevelopment) of the corpus callosum, involving either the splenium and posterior body or the rostrum[43]. These anomalies suggest that the disruption of neural migration that is associated with myelomeningocele is prolonged into the second trimester of pregnancy, as the corpus callosum develops in weeks 8–20 of gestation[49]. Quantitative studies of the corpus callosum show marked volume and integrity differences between individuals with myelomeningocele and unaffected controls; particularly pronounced differences occur posteriorly when the corpus callosum is hypogenetic or hypoplastic[50]. Reduced integrity has also been shown in the genu but not in the anterior commissure in affected patients[51]. Some evidence supports the theory that corpus-callosal defects are associated with closed spinal dysraphism[52].

Secondary consequences of myelomeningocele might also include hydrocephalus. This condition arises primarily from an obstruction of cerebrospinal fluid flow at the level of the fourth ventricle of the brain, but other contributing factors include aqueductal stenosis, venous haemodynamics and ependymal denudation. Cortical reorganization occurs around the area of ventricular dilatation. On the basis of quantitative studies, the frontal regions seem to be enlarged and posterior cortical regions reduced in volume compared with controls[53]. Hydrocephalus stretches the white matter, and this is most apparent in the thinned (hypoplastic) appearance of the corpus callosum[54]. Diffusion tensor imaging of white matter structures shows that the integrity of the long association fibre tracts connecting the posterior and anterior brain regions is consistently reduced in affected individuals relative to typically developing controls[55,56]. Using the midbrain as a reference point of origin, it has been shown that individuals with myelomeningocele have a greater reduction in posterior white matter integrity than in frontal pathways, especially in association with tectal beaking[57] (FIG. 3d–i).

Hydrocephalus is accompanied by cognitive and motor abnormalities, the severity of which is linearly associated with the amount of white matter impairment[58]. The aberrant volumes of frontal versus posterior regions are associated with reductions in IQ and in dexterity of the fingers[59]. In addition, the specific contributions of the Chiari II malformation to cognitive and motor outcomes might be underestimated. The Chiari II malformation is associated with eye movement difficulties, as well as problems with the precision and timing of motor movements and rhythmicity[60]. Attention deficit is also common in patients with myelomeningocele, reflecting problems with the midbrain and parietal lobe attention systems, which are involved in orienting to salient features of the environment and in arousal. Additionally, tectal anomalies correlate directly with the level of orienting deficits[61]. By contrast, motor skills that are learned through practice, as well as sustained attention and persistence, are generally preserved after the initial phases of learning, possibly reflecting a lower level of impairment in the frontal–striatal regions and basal ganglia[60]. The corpus callosum anomalies in individuals with myelomeningocele are associated with reduced interhemispheric communication and difficulties in integrating information received via language, reading and social interactions[62].

These neurocognitive difficulties can be observed as early as 6 months of age[63] and affect patients throughout their lives. They lead to difficulties in learning to construct and assimilate information (assembled processing), in contrast to the relative strengths of affected individuals in associative and procedural learning (associative processing)[64] (TABLE 1). The characteristic cognitive strengths and weaknesses associated with myelomeningocele are highly variable and poorly reflected by IQ scores. Intellectual disability

Table 1 | **Modal cognitive strengths and weaknesses in spina bifida**

Mode	Strengths in associative processing	Weaknesses in assembled processing
Perception	Categories and faces	Representations
Language	Vocabulary and grammar	Constructing meaning
Reading	Decoding	Comprehension
Mathematics	Number facts	Algorithms
Behaviour	Sociability	Adaptation

affects ~20–25% of people with myelomeningocele and often only after complications such as hydrocephalus. A study in the United States has shown that, relative to non-Hispanic individuals, Hispanic individuals have a greater frequency of impaired cognitive outcome, and that this is associated with a higher prevalence of upper-level myelomeningocele defects and of low socio-economic status[46].

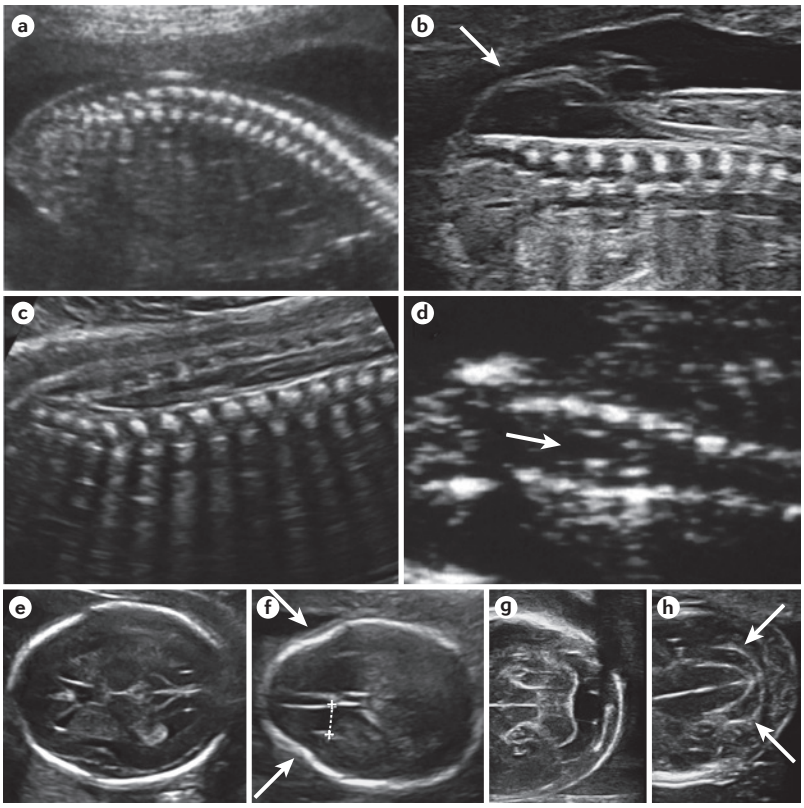

Figure 4 | **Myelomeningocele and associated cranial signs on ultrasonography.** Diagnostic ultrasonography images of normally developing fetuses and fetuses with myelomeningocele. Compared with the regular, parallel vertebrae covered with skin in a normal fetus (part **a**), the spine is protruding from the vertebral column in myelomeningocele (arrow, part **b**). The low spinal view of a normal fetus (part **c**) shows the cauda equina within the vertebral canal, whereas in spina bifida, a protruding meningeal cyst is visible (arrow, part **d**). In a typically developing fetus, the skull has a regular, smooth frontal appearance (part **e**). By contrast, cranial signs that accompany myelomeningocele include the lemon sign, which is due to scalloping of the frontal bones (arrows, part **f**). Of note, the size of the anterior horn is also marked in part **f**. Compared with the dumb-bell shape of the unaffected fetal cerebellum (part **g**), the banana sign seen in myelomenigocele is characterized by a convex-shaped cerebellum (arrows, part **h**).

Diagnosis, screening and prevention

Biochemical diagnosis and screening

Prenatal diagnosis first became possible in the early 1970s and was based on an elevated concentration of α-fetoprotein in the amniotic fluid of women carrying fetuses with anencephaly or myelomeningocele[65,66]. Subsequently, the presence of acetylcholinesterase in amniotic fluid was also shown to be diagnostic[67]. Although α-fetoprotein measurement in amniotic fluid samples might be useful for high-risk pregnancies, the 1% chance of miscarriage following amniocentesis has limited the general application of this test. However, it has been found that α-fetoprotein levels are elevated in serum samples from mothers carrying fetuses with myelomeningocele[68], and this finding formed the basis of subsequent population-screening approaches[69]. Now, with second-trimester anomaly scanning becoming routine, biochemical screening is becoming redundant, as ultrasonography offers greater sensitivity and specificity. Currently, the main indication for biochemical screening is maternal obesity, which can impair detailed ultrasound examination of the fetal anatomy.

Sonographic diagnosis

In parallel with the development of biochemical assays in the 1970s, improvements in ultrasonography facilitated non-invasive diagnosis of myelomeningocele and other NTDs[70]. Today, the fetal spine can be examined by ultrasonography in the sagittal, axial and coronal planes from late in the first trimester onwards, providing the principal and most accurate mode of prenatal diagnosis. For reliable detection of myelomeningocele, detailed systematic examination is required in all three planes along the entire length of the spine, from cervical to sacral. This degree of careful examination can detect the majority of cases of myelomeningocele, whereas skin-covered (closed) lesions are rarely identified *in utero*. Views of the normal spine juxtaposed with myelomeningocele, to demonstrate the sonographic differences, are shown in FIG. 4. The spinal lesion is most readily identified when examined in the sagittal plane (FIG. 4a,b), particularly if the lesion is associated with a meningocele or myelomeningocele, when the cystic extension is often visible from the posterior aspect of the spine (FIG. 4c,d). The presence of neural tissue within the sac can often be seen, although ultrasonography cannot reliably exclude the presence of neural tissue. In addition to spina bifida, associated spinal distortions — in varying degrees from virtually none to severe kyphoscoliosis — can also be observed by ultrasonography.

Table 2 | **Detection rate of cranial markers of spina bifida by ultrasonography**

Study	Abnormality*			
	Lemon sign	Small, banana-shaped cerebellum	Ventriculomegaly	Microcephaly
Nicolaides *et al.*[73]	100% (*n* = 54)	95% (*n* = 21)	62% (*n* = 70)	86% (*n* = 66)
Campbell *et al.*[230]	100% (*n* = 26)	95% (*n* = 26)	65% (*n* = 26)	54% (*n* = 26)
Nyberg *et al.*[74]	93% (*n* = 14)	NR	NR	NR
Thiagarajah *et al.*[76]	100% (*n* = 16)	100% (*n* = 16)	69% (*n* = 16)	63% (*n* = 16)
Van den Hof *et al.*[79]	98% (*n* = 107)	96% (*n* = 107)	NR	NR
Bahlmann *et al.*[77]	88.6% (*n* = 588)	97% (*n* = 588)	46% (*n* = 588)	70% (*n* = 588)
Total	91% (*n* = 815)	97% (*n* = 758)	49% (*n* = 700)	71% (*n* = 696)

NR, not reported. *Percentage of abnormalities detected per total number (*n*) of fetuses with spina bifida.

Several cranial features are associated with myelomeningocele, including a disproportionately small biparietal diameter for gestational age[71] and varying degrees of ventriculomegaly, the latter of which might occur in almost all affected fetuses by the third trimester but is present in up to 70% of spina bifida cases in the second trimester[72]. In the late 1980s, the 'lemon' and 'banana' signs (FIG. 4e–h) were described[73]. The lemon sign refers to a loss of the convex outward shape of the frontal bones, with mild flattening (FIG. 4e,f), and is present in almost all fetuses with myelomeningocele at between 16 weeks and 24 weeks of gestation (TABLE 2). After 24 weeks, the lemon sign is detected in only 30–50% of affected fetuses[74–77]. The banana sign refers to the shape of the cerebellum (FIG. 4g,h) and is thought to be due to tethering of the spine with downward traction on the cerebellum (the Chiari II malformation); it can be detected from 14 weeks onwards[78]. Cerebellar abnormalities are present in 95% of fetuses with myelomeningocele, irrespective of gestational stage. However, the banana sign is detected most commonly before 24 weeks of gestation (in 72% of all fetuses with myelomeningocele). In later pregnancy, the cerebellum cannot be detected on ultrasonography in ~80% of affected fetuses, and thus the banana sign is not a reliable indicator[79].

These cranial signs have been important aids to prenatal diagnosis, as the head is examined routinely in all fetuses in the second trimester. Detailed spinal examination might be compromised by fetal position or other technical factors such as maternal habitus. However, detection of the cranial abnormalities should be an indication to ensure that detailed examination of the spine needs to be undertaken, and in many clinics this results in tertiary referral. Routine second-trimester ultrasonography now detects ~90–98% of fetuses with myelomeningocele in countries that offer second-trimester anomaly scanning[80] (TABLE 3). These screening methods are more than 10 years old, and obesity has become increasingly more common in the obstetric population since their development. However, the vast improvements in ultrasonography over time ensures that routine fetal-anomaly scanning will continue to have a considerable impact on the prenatal detection of NTDs[81]. According to the UK National Ultrasound Screening Programme, the minimum standard for the detection of this anomaly following routine second-trimester anomaly scanning should be more than 90%[82].

Following the identification of spina bifida, detailed examination of the fetus is performed to look for other signs that might indicate an associated chromosomal or genetic syndrome, and to seek evidence of neurological damage, such as talipes or a dilated renal tract. Karyotyping is offered when other abnormalities are detected or when other risk factors (for example, advanced maternal age) might suggest an associated chromosomal abnormality[77,83]. Prediction of the spinal level of the lesion is advantageous, as this might determine the prognosis. Indeed, three-dimensional ultrasonography in one small study has been shown to detect the defect level to one spinal segment in 86% of cases[84]. However, the anatomical level of the lesion frequently does not correspond to the functional level. As a consequence, ultrasonography was not found to be predictive for postnatal mobility or intellectual function[85].

Table 3 | **Detection of spina bifida at the time of a routine ultrasonograpy**

Study	Study period	Location	Spinal abnormalities*
Smith and Hau[231]	1989–1994	Scotland	92% (*n* = 87)
Boyd *et al.*[232]	1991–1996	Oxford, UK	98% (*n* = 46)
Shirley *et al.*[233]	1986	Hillingdon, UK	100% (*n* = 3)
Chitty *et al.*[234]	1988–1989	Luton, UK	100% (*n* = 5)
Luck[235]	1988–1991	Ascot, UK	100% (*n* = 2)
Papp *et al.*[236]	1988–1990	Hungary	91% (*n* = 44)
Total	NA	NA	94% (*n* = 187)

NA, not applicable. *Percentage of spinal abnormalities detected per total number (*n*) of fetuses with spina bifida.

Prevention

The prevention of NTDs by maternal folic acid supplementation has been heralded as a modern public health success[86]. Nearly 40 years ago, Smithells *et al.*[87] found that diets and postpartum blood levels of women who had a pregnancy affected by NTD were mildly deficient for selected micronutrients, including

Box 3 | **Surgical treatments for myelomeningocele**

- Choroid plexus coagulation: the cerebrospinal fluid (CSF)-producing choroid plexus is coagulated endoscopically to prevent further CSF production, which otherwise exacerbates the hydrocephalus.
- Ventriculoperitoneal shunt: a shunt is inserted to drain CSF from the brain ventricles into the peritoneal cavity.
- Ventriculostomy: a small perforation is made in the thinned floor of the third ventricle, allowing movement of CSF out of the blocked ventricular system and into an adjacent space that is normally filled with CSF.

folate. Administration of a folic acid-containing multivitamin supplement reduced the risk of NTD recurrence in women with a previously affected pregnancy[88]. Subsequently, the UK Medical Research Council randomized clinical trial of NTD recurrence[89], a randomized trial of NTD first occurrence[90] and a number of observational epidemiological studies all provided evidence that folic acid supplements can prevent NTDs from occurring during pregnancy. Now, women at high risk, including those with a previous history of an NTD-affected pregnancy, are recommended to take 4 mg of folic acid per day while planning a pregnancy, whereas those at low risk are advised to take 0.4 mg per day[86].

Concerns about the effectiveness of voluntary supplementation have led to policy decisions in many countries, including the decision to fortify staple foods with folic acid. Mandatory folic acid fortification of cereal grain products in the United States began in January 1998 and has been associated with an ~25% reduction in the prevalence of NTDs[91]. Implementation of mandatory fortification programmes elsewhere — such as in Chile[92], Costa Rica[93], Canada[94], South Africa[95] and Saudi Arabia[96] — has been associated with similar or even greater reductions (>50%) in the prevalence of NTDs, and particularly that of spina bifida. Brazil[97] and Peru[98], by contrast, did not report a reduced NTD prevalence after fortification programmes. The relative reduction in prevalence seems to be roughly correlated with the magnitude of the initial prevalence of NTDs. Some countries, such as Australia, have also observed reductions in NTD prevalence after implementing programmes of voluntary folic acid supplement use or fortification[99]. Whether to establish mandatory fortification programmes in European countries remains much debated. Some scientists have questioned whether these programmes should go further towards reaching susceptible pregnancies[100], whereas others have expressed the need to balance the benefits of NTD prevention with possible risks (such as potential masking of vitamin B12 deficiency or enhanced growth of pre-malignant lesions) for other sections of the population[101,102].

Management

The management of myelomeningocele traditionally involves surgery within 48 hours of birth. The child's back is closed to minimize the risk of ascending infection, which can otherwise result in meningitis. However, an earlier intervention involving fetal surgery has now been implemented in a number of centres, with promising results.

Postnatal surgery and management

Newborn babies with myelomeningocele are best managed following baseline imaging studies of the central nervous system, and subsequent serial head measurements to assess the velocity of head growth and the need for shunting. Almost all newborns with thoracic-level lesions need a ventriculoperitoneal shunt (BOX 3), whereas ~85% of patients with a lumbar-level lesion and ~70% with a sacral-level lesion require this intervention[103]. Over the past 5 years, combined endoscopic third ventriculostomy and choroid plexus coagulation (BOX 3) has become an alternative treatment for hydrocephalus associated with spina bifida in selected cases[104]. Radiological evidence of the Chiari II malformation is present in most affected individuals, and clinically symptomatic hindbrain herniation can affect up to 30% of patients. Symptoms of this herniation include apnoea, swallowing difficulties and stridor in a newborn baby, or headache, quadriparesis, scoliosis, and balance and/or coordination issues in an older child months or years later. In severe cases, posterior fossa decompression surgery is indicated[105].

Orthopaedic deformities are usually treated shortly after birth, but they require long-term follow-up. Patients are also monitored by ultrasonography and urodynamic studies to detect urological complications resulting from abnormal neurological control of bladder function. Possible complications include urinary retention with overflow and ureteric reflux, which can lead to recurrent urinary tract infections and, ultimately, deterioration of renal function. Bladder and urinary tract management often involves a combination of clean, intermittent catheterization, pharmacological agents and surgery[106]. Bowel function is not an issue in newborns, but older children require bowel management treatments such as suppositories, laxatives, or antegrade colonic or traditional enemas[107,108].

Medical management is best provided through regular assessments by a multidisciplinary team, including a nurse specializing in the care of children with multiple handicaps, a paediatric neurosurgeon, a urologist, an orthopaedic surgeon, a physical therapist and a social worker. Other subspecialists (for example, a psychologist) can be involved if required. Communication between the multidisciplinary team members and the patient's primary physician is vital. Additional issues that might need to be addressed by the team include neurobehavioural development, mobility and means of locomotion, weight maintenance, skin care and the avoidance of latex sensitization.

Fetal surgery

The rationale for fetal surgery[109] is that damage to the exposed spinal cord progresses during gestation. Hence, early repair of the lesion *in utero* might prevent continuing damage and improve clinical outcome. Additionally, myelomeningocele repair arrests the

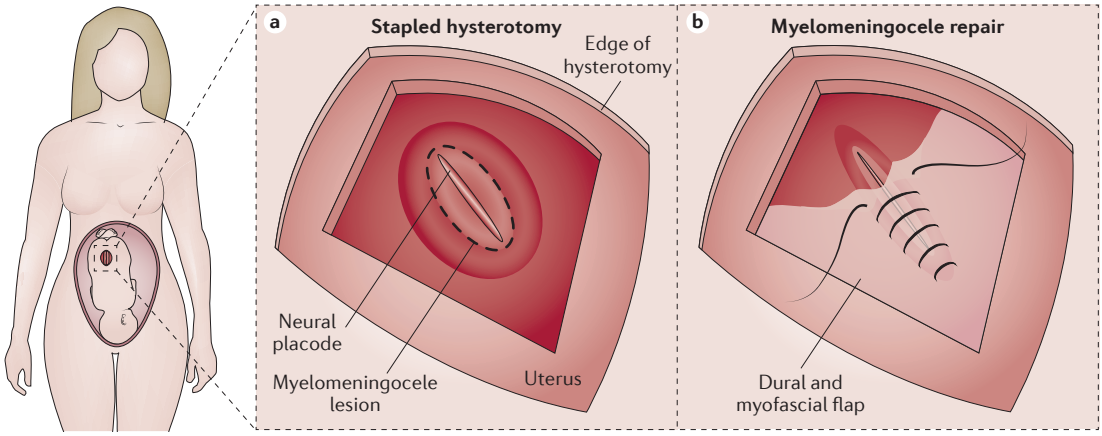

Figure 5 | **Fetal surgery for spina bifida.** When a human fetus with spina bifida reaches 22 weeks of gestation, the mother and fetus can undergo surgery to repair the fetal spinal lesion. First, a hysterotomy is made in the mother by a uterine stapler, exposing the myelomeningocele lesion and neural placode (part **a**). This is followed by closure of the myelomeningocele lesion using a dural and myofascial flap (part **b**).

leak of cerebrospinal fluid from the lesion, enabling the reversal or resolution of hindbrain herniation[110–112].

Pregnant mothers carrying a fetus with diagnosed myelomeningocele who consider *in utero* surgery undergo extensive prenatal testing. This testing includes an obstetric evaluation, screening for genetic or chromosomal syndromes, and ultrasonography of the fetus to assess leg function, identify defects including talipes and determine the spinal level of the vertebral-arch anomalies. Fetal echocardiography is used to identify any coexisting cardiac defects, and ultrafast MRI is used to detect the features of the Chiari II malformation, including hindbrain herniation and other brain anomalies, and to determine the presence or absence of hydrocephalus[113].

Fetal myelomeningocele surgery is conducted according to an intraoperative and postoperative management algorithm[114]. The surgery involves maternal laparotomy (abdominal wall incision), after which a uterine stapling device is used to create a 6–8 cm hysterotomy (uterine incision) of sufficient size to expose the fetal lesion (FIG. 5). Intraoperative echocardiography is used throughout the procedure to monitor fetal heart function[115]. Closure of the defect follows a standard procedure similar to that used postnatally: the cystic membrane is excised, meningeal attachments to skin and soft tissues are mobilized, and the neural placode is separated from surrounding tissue and positioned in the spinal canal. If possible, the dura is identified, reflected over the placode and closed with sutures. Paraspinal myofascial flaps are created and closed in the midline. Skin flaps are then used to complete the repair (FIG. 5), but if the skin cannot be closed primarily, the procedure is completed using an acellular human dermis graft.

Successful *in utero* spina bifida repair was first reported in 1998 (REFS 116,117). Clinical use grew rapidly thereafter, with promising results, but without compelling proof of safety or efficacy[110,111]. To gain this evidence, a prospective randomized clinical trial, called the Management of Myelomeningocele Study (MOMS), was initiated in 2003. The objective of MOMS was to evaluate fetal and maternal outcomes following intrauterine repair of myelomeningocele between 19 weeks and 25 weeks of gestation, compared with standard postnatal neurosurgical repair[114]. The MOMS clinical centres — The Children's Hospital of Philadelphia (Pennsylvania, USA), Vanderbilt University (Nashville, Tennessee, USA) and the University of California, San Francisco (USA) — all adopted uniform criteria for patient inclusion and exclusion, and identical protocols for prenatal and postnatal patient care. The trial had two primary outcome measures: first, a composite of fetal or neonatal death or the need for placement of a ventriculoperitoneal shunt by the age of 12 months; and second, a composite score of mental development (using Bayley Scales of Infant Development II (REF. 118)) and motor function at the age of 30 months. A number of other (secondary) neonatal and maternal outcome measures were examined, including premature birth, which is a known complication of *in utero* surgery. Follow-up evaluation of children and mothers was conducted by a team of paediatricians and psychologists who were unaware of the study group assignments, thereby ensuring blinding of the final analysis.

Enrolment into MOMS was stopped, on the basis of the efficacy of fetal surgery, by the Data Safety and Monitoring Board in December 2010. By this time, 183 of the planned 200 patients had been randomly assigned. The MOMS trial findings confirmed the results of earlier nonrandomized evaluations of fetal myelomeningocele repair. Specifically, MOMS found that fetal surgery led to a significant reduction in the frequency of ventriculoperitoneal shunt placement at 12 months of age (fetal-surgery group: 40%; postnatal-surgery group: 82%) and an improvement in overall neuromotor function at 30 months of age. Of the children who received fetal surgery, 42% were walking independently at 30 months of age, compared with only 21% in the postnatal-surgery group. This result was despite the fact that, on average, individuals in the fetal-surgery

Box 4 | **Inclusion and exclusion criteria for fetal repair of myelomeningocele**

Inclusion criteria

- Maternal age of at least 18 years
- Gestational age at randomization of between 19 weeks 0 days and 25 weeks 6 days
- Normal karyotype
- S1-level lesion or higher
- Confirmed hindbrain herniation on prenatal ultrasound and MRI

Exclusion criteria

- Multiple-gestation pregnancy*
- Additional fetal anomalies unrelated to spina bifida*
- Fetal kyphosis ≥30 degrees*
- Placenta previa*
- Incompetent and/or short (<20 mm on ultrasonographic scan) cervix‡
- History of spontaneous early birth (singleton delivery at <37 weeks of gestation) ‡
- Maternal–fetal rhesus group isoimmunization‡
- Insulin-dependent pregestational diabetes‡
- Obesity defined by a body mass index of ≥35‡
- Positive for HIV, hepatitis B virus or hepatitis C virus‡
- Uterine anomaly‡
- Another serious maternal medical condition‡
- Psychosocial limitations‡
- Lack of support‡
- Inability to comply with travel and follow-up‡

*Fetal or pregnancy-related factor. ‡Maternal factor[114].

group had higher and more severe myelomeningocele lesions than those in the postnatal-surgery group. Significantly less hindbrain herniation occurred in the fetal-surgery group than in the postnatal-surgery group. However, premature birth was significantly more common after fetal surgery than in the postnatal-surgery group. In addition, ~25% of women whose babies underwent fetal surgery showed evidence of thinning of the uterine wound at the time of delivery, and 10% showed tissue edge separation at the hysterotomy site (9% showed partial separation and 1% complete separation), although none had a hysterotomy rupture.

As a result of the MOMS trial, three options are now available for women carrying a fetus diagnosed with myelomeningocele at less than 24 weeks of gestation. They can choose to terminate the pregnancy; to continue the pregnancy with delivery by near-term caesarean section followed by postnatal repair; or to undergo prenatal surgery, provided that the eligibility criteria are satisfied (BOX 4). Financial modelling of data from the MOMS trial has identified a health care saving of more than $2 million for every 100 cases of fetal myelomeningocele repair undertaken[119]. However, long-term follow-up of children who receive fetal repair will be crucial to assess whether the early benefits are enduring. To this end, the MOMS trial patients will now be followed up to the age of 6–10 years in a US National Institutes of Health-funded study. The clinical experience with fetal myelomeningocele repair during the past 3 years has shown comparable results to the MOMS trial[120]. Institutional guidelines have also been established, and it is clear that to optimize patient safety and outcome, fetal myelomeningocele surgery should be conducted only in high-volume fetal-surgery centres that follow a standardized patient care protocol[121]. The North American Fetal Therapy Network has established a data registry to collate the outcomes for patients who have the surgery.

Quality of life

Spina bifida has a pervasive impact on the physical, neurocognitive, psychological and social functioning of affected individuals[122–124] (FIG. 6).

Health-related quality of life

Children and adolescents with spina bifida have a lower health-related quality of life than both individuals without spina bifida and children with other chronic health conditions. These differences tend to be stable across age groups, sexes and geographical locations, and they seem to continue throughout a patient's life[125,126]. Although measures of severity, such as lesion level, continence status and outcomes of various surgical procedures, tend not to correlate with health-related quality of life[126,127], other factors do have a correlation, particularly the presence of shunted hydrocephalus and lack of mobility[128,129]. Other robust predictors of health-related quality of life include socio-economic status, pain levels, parenting stress and other family factors[126,130].

Psychosocial adjustment

During late childhood, individuals with spina bifida tend to have higher levels of depressive symptoms and lower levels of self-esteem than unaffected individuals[131–133]. They also have social difficulties, including social immaturity and passivity, having fewer friends and social contacts outside school, and having fewer romantic relationships during adolescence[131,134,135]. Most of these difficulties seem to be maintained into young adulthood[132]. Children and adolescents with spina bifida also depend more on adults for guidance, show less intrinsic motivation at school and exhibit less behavioural autonomy at home. They express their own viewpoints less frequently than typically developing individuals during family interactions[131,136–138].

Family functioning

Functioning of the families of children and adolescents with spina bifida[139] is characterized by a resilience–disruption model[140]. This model is based on the concept that a child with spina bifida might disrupt some normative family functions, but many families exhibit considerable resilience, are able to adapt to such disruption and often exhibit similar levels of conflict as families with only typically developing children. Approximately 10–15% of families affected by spina bifida exhibit clinical levels of family dysfunction[141,142], but these rates are lower than the 35% of clinical family dysfunction found among families containing children with cerebral palsy[142]. The combination of lower socio-economic status and parenting a child with spina bifida further increases the risk of lower levels of family cohesion, which supports a cumulative risk model for these families[143].

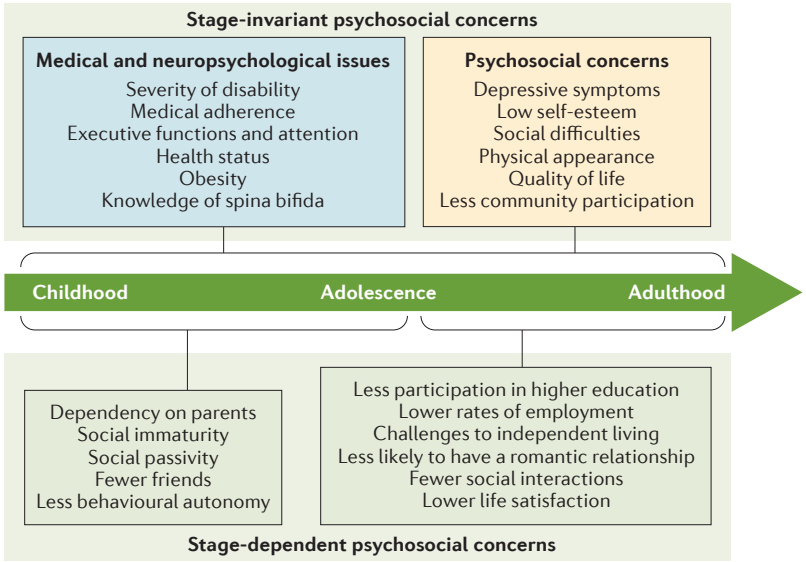

Figure 6 | **Quality-of-life concerns across developmental stages in patients with spina bifida.** Schematic representation of the main quality-of-life concerns for individuals with spina bifida.

Studies of marital functioning among parents of children with spina bifida have yielded mixed results[144–146], although the quality of the relationship before the affected child was born has emerged as an important predictor of subsequent family adjustment. A meta-analysis of 15 studies revealed a consistent negative impact of spina bifida on the psychological adjustment of parents, with an effect size that was larger, but more heterogeneous, for mothers ($d = 0.73$) than for fathers ($d = 0.54$)[147]. The study also showed negative effects on parental stress levels and parenting quality[138,148]. Typical findings are that parents of children with spina bifida feel more isolated, are less satisfied and/or competent as parents, are less adaptable to change and hold less optimistic views about the future than parents of typically developing children[145,149,150]. This particularly applies to parents who are single, are at the older end of the parental age range, experience social isolation, or are from a non-white background or a low socio-economic background[143,151]. Siblings of children with spina bifida benefit in their personal adjustment when they experience positive family attitudes to spina bifida, greater overall family satisfaction and lower levels of sibling conflict[152].

Adult outcomes

The mortality rate among young people with spina bifida is ~1% per year between 5 and 30 years of age, with the rate being highest among those with the highest-level lesions[153,154]. Among survivors, the quality of the individual's health tends to decline from adolescence to young adulthood, presumably owing to difficulties in navigating the transition to adult health care[155–157]. Similar to their younger counterparts, emerging adults with spina bifida are at increased risk of depressive symptoms and anxiety[129,158]. They are, however, less likely to engage in risky behaviours such as alcohol use and multiple sexual partners, possibly owing to their lower rates of social integration[159]. Regarding educational and vocational outcomes, 41–56% of young adults with spina bifida go to college compared with 66% of typically developing young people[128,153,160,161]. Moreover, recent studies report that only 36–48% of individuals with spina bifida are in full-time or part-time employment[128,156,161–163], which is significantly lower than the rate for typically developing young people (which is 75%[160,161,164]) and for those with other chronic conditions (which is 68% for individuals with asthma and 78% for individuals with cancer[156,165]). Moreover, half of individuals with spina bifida who work have part-time positions, and their annual salary is therefore below the US national average[128].

With respect to relationship quality, 43–77% of adults with spina bifida live with their parents[128,153]. Just over half (52–68%) have had a romantic relationship[128], which is less than in typically developing young adults[161,166]. The lowest level of life satisfaction is in the areas of romantic relationships, employment and financial independence[128]. Parents of young people with spina bifida are less likely to discuss issues of sexuality with their children[134,167], and most affected individuals have an inadequate level of knowledge in this area[168]. The high rate of obesity in this population (rates tend to be >40% (REF. 169)), coupled with continence issues, probably undermine these young adults' efforts to have romantic relationships[169,170]. Moreover, participation in leisure and recreational activities tends to be low, with >50% failing to participate[171]. The most common barriers are lack of motivation, lack of information and time constraints[171]. Younger individuals and those without shunts tend to participate more than older and more-impaired individuals[172]. Generally, the best predictors of successful navigation of young adult milestones seem to be condition related (an absence of hydrocephalus, and good mobility[128]), neuropsychological (executive functioning[161]), personality based (intrinsic motivation[161]), familial (socio-economic status and parental intrusiveness[161]) and logistical (transportation and accessibility[173]). Other influential factors include financial aspects (concerns about lack of health insurance[174]), lack of job training and vocational rehabilitation services, discrimination during employment, physical-appearance stigmas and early-childhood socialization problems[170,175,176].

Outlook

Spina bifida affects individuals, their families, medical science and society in a variety of ways. Looking forward, it is exciting to discern a number of areas in which our understanding of this multifaceted condition is likely to advance, both by enhancing our ability to promote primary prevention and by improving the lives of individuals who have spina bifida.

Genetic basis

For spina bifida, like many other diseases, efforts to unravel the cause of the disorder will be enhanced by the application of recently developed high-throughput genomic and epigenomic technologies. Exome sequencing is already being applied on a small scale[177]. However, even if causal genetic variants are identified in individuals

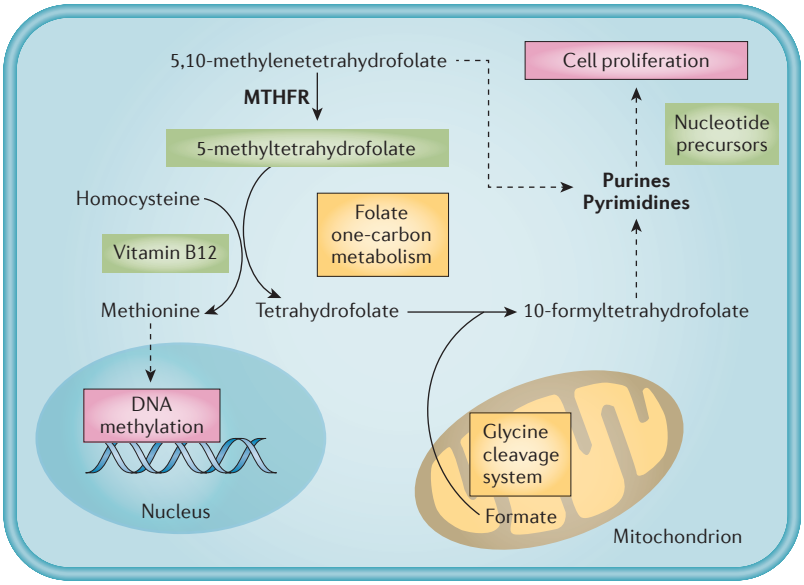

Figure 7 | **Folate metabolism and possible interventions.** Maternal supplementation with folic acid prevents many cases of spina bifida, most probably through its regulation of epigenetic modifications (methylation) and/or cell proliferation (through a role in the synthesis of purines and pyrimidines) in the embryo, although the exact mechanism is incompletely understood. However, defects in enzymes involved in these pathways might mean that folic acid supplementation alone is inadequate and point to the need to supplement with other metabolites (green boxes). Thus far, mutations in the genes encoding several enzymes involved in the folate one-carbon metabolism pathway (especially in the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR); maternal and fetal mutations) and the glycine cleavage system (which produces formate in the mitochondria; fetal mutations only) have been definitively associated with increased risk of spina bifida. Solid arrows indicate the key metabolic reactions. Dashed arrows indicate metabolic pathways that involve multiple reactions.

or families with spina bifida, many are likely to be irrelevant to spina bifida causation on a wider scale. Moreover, genetic risk might be imparted by non-coding DNA variants (such as enhancer polymorphisms) and specific epigenetic signatures[178,179], neither of which is detected by exome sequencing. What is needed is a general and unbiased approach, at the level of multinational consortia, to identify genomic and epigenomic alterations within groups of individuals with spina bifida (and other NTDs) compared with unaffected controls[179]. With continued reductions in the cost and increases in the speed of high-throughput technologies, the application of more comprehensive and integrated 'omics' methodologies, including protein and metabolite detection and quantitation, seems likely to be implemented in the coming years[180].

Preventive action of folic acid

The mechanism by which folic acid prevents spina bifida and other NTDs remains unclear, and experimental studies will be aimed at elucidating this important aspect of primary prevention (FIG. 7). Exogenous folic acid might enhance embryonic cell proliferation through stimulation of pyrimidine and purine synthesis. This hypothesis is supported by the finding that embryonic cells undergo disordered proliferation in several mouse models of NTDs[181]. Another possibility is that folic acid enhances the methylation of key macromolecules, including DNA. Thus, by interfering with the epigenetic regulation of early nervous system development, the compound might affect embryonic gene expression[178,179]. A more controversial hypothesis is that sometimes folic acid might not protect from neural tube closure defects, but rather might exacerbate these problems, thereby worsening fetal outcomes and leading to miscarriages[182]. Such a detrimental effect, termed terathanasia, could in principle account for a reduced NTD prevalence in later pregnancy, as most affected pregnancies would have been lost earlier. In mice, multigenerational treatment with high-dose folic acid increased the frequency and severity of NTDs in three mutant mouse strains[183]. However, in another mouse strain, NTDs were reduced by folic acid administration and increased by dietary folate deficiency[184], consistent with a role of these compounds in true primary prevention. When we are able to identify specific subgroups of human spina bifida, for example from their genetic risk factors, it might be possible to determine whether folic acid supplementation also has these opposing actions in humans.

Prevention by other metabolites

Finding the means to prevent more cases of spina bifida is a priority for future research and public health implementation. Folic acid food fortification is likely to be extended to countries where this is not currently practised[100], and supplements containing vitamin B12, a cofactor in folate one-carbon metabolism, might further reduce the frequency of NTDs[185]. In addition, apparent 'folate non-responsiveness' is being increasingly observed, as women continue to experience spina bifida-affected pregnancies despite taking folic acid supplements. Some NTDs might fail to respond to exogenous folic acid owing to defects in the folate transport system (that is, the metabolic enzymes required to transfer one-carbon units to key downstream metabolites); such defects could prevent folic acid from being metabolized. In this case, supplementation with alternative folates, such as 5-methyltetrahydrofolate[186], or with key downstream molecules such as nucleotide precursors[187] might enhance primary prevention (FIG. 7).

Alternatively, some NTDs can arise from an embryonic defect that cannot be corrected by altering folate one-carbon metabolism, and quite different preventive strategies might be required. Prominent among these strategies is addition of the small molecule inositol, which is essential for a number of intracellular signalling pathways and is a building block for membrane phospholipids. Inositol supplementation can prevent NTDs in a folate-nonresponsive mouse strain[188], and has proved to be well tolerated and associated with normal fetal outcomes in a group of women whose fetuses are at high risk of developing spina bifida[189]. A pilot randomized clinical trial of combined inositol and folic acid supplementation has recently been completed in the UK[190].

Fetal surgery with stem cells

Building on the success of the MOMS clinical trial, studies to evaluate the effect of introducing stem cells into the open spinal cord at operation are being initiated. If stem cells are able to replace damaged or dead cells in

the lesion, neurological function might be enhanced after birth. To date, stem cells have been transplanted into rat and sheep fetuses with myelomeningocele. These studies used mesenchymal stem cells, neural stem cells and skin-derived induced pluripotent stem cells that were treated to enhance neural crest cell differentiation, and all three cell types survived for variable periods after transplantation[191–193]. Importantly, biodegradable tissue scaffolds have been successfully inserted in the lesion during the *in utero* operation, enabling cells to be seeded and grown on the scaffold prior to transplant[193]. However, using autologous (host-derived) amniotic fluid-derived stem cells for transplantation, to minimize the risk of graft rejection, might prove problematic. Indeed, these cells, when isolated from human fetuses with spina bifida, fail to deposit collagen type I and show lower collagen-related gene expression than cells from the amniotic fluid of normal fetuses[194]. A further stem cell-related advance has been the demonstration that autologous bone marrow-derived stem cells can be used in combination with a tissue scaffold to enable bladder tissue engineering. This strategy might replace enterocystoplasty (in which bowel wall is used to reconstruct the bladder), a procedure commonly performed in children with myelomeningocele. Clearly, a great deal of work will be needed, both *in vitro* and in animal models, to develop optimal protocols for both efficacy and safety before stem cell transplants can be considered in human fetuses. Furthermore, to maximize the effectiveness of any potential *in utero* treatment, early sonographic diagnosis will be needed, and this will require the development of routine screening programmes delivered at ~12 weeks of gestation.

Psychosocial interventions

Several domains in the lives of individuals with spina bifida need further research. This is exemplified by the current lack of family-based intervention studies for the families of young people with spina bifida[139], in contrast to the extensive literature in this area for other chronic physical conditions (for example, type 1 diabetes mellitus). Although few randomized clinical trials have been reported in any of the salient psychosocial domains (quality of life, social skills, independent decision making and depressive symptoms), one small randomized study of adults with spina bifida found that goal management training reduced anxiety and psychological distress[195]. A manualized summer camp-based intervention has also been developed that targets independence and social skills among children, adolescents and young adults with spina bifida. The intervention included collaborative (that is, parent and camper) goal identification, group sessions consisting of psycho-education and the acquisition of cognitive tools, and goal monitoring by camp counsellors. Goals for each camper included a medically related goal (for example, catheterizing independently) and a social goal (for example, making a new friend during camp). Statistically significant gains were found both in individualized goals and in the independent management of spina bifida-related responsibilities, with medium effect sizes[196]. Such gains were maintained at a 1-month follow-up, and the findings have since been replicated with larger effect sizes[197]. Progress towards the objective evaluation of such interventions could considerably improve the lives of individuals with spina bifida.

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