Spina Bifida

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Abstract

Spina bifida is a birth defect in which the vertebral column is open (bifid), often with spinal cord involvement. Clinically most significant is myelomeningocele (MMC; open spina bifida) in which the spinal neural tube fails to close during embryonic development. The exposed neural tissue degenerates *in utero*, resulting in neurological deficit that varies with level of the lesion. Occurring in around 1 per 1000 births worldwide, MMC is one of the commonest congenital malformations, yet its causation is largely unknown. The genetic component of MMC is estimated at 60-70% but few genes have yet been identified, despite much information from mouse models. Non-genetic risk factors include reduced folate intake, maternal anticonvulsant therapy, diabetes mellitus and obesity. Primary prevention by peri-conceptional folic acid has been demonstrated in clinical trials, leading to food fortification programmes in many countries. Prenatal diagnosis is by ultrasound enabling termination of pregnancy. Individuals who survive to birth have their lesions closed surgically, with subsequent management of associated defects, including the Chiari II malformation, hydrocephalus, and urological and orthopaedic sequelae. Fetal surgical repair of MMC has been associated with improved early neurological outcome compared with postnatal operation. MMC affects quality of life during childhood, adolescence, and into adulthood, posing a challenge for individuals, families and society as a whole.

0. Introduction

Spina bifida is a congenital malformation in which the spinal column is split (bifid) as a result of failed closure of the embryonic neural tube, during the fourth week post-fertilization. In its commonest and most severe form, myelomeningocele (MMC; also termed open spina bifida or spina bifida aperta), the spinal cord is open dorsally, forming a placode on the back of the fetus or newborn baby that frequently rests on a meningeal sac (then named spina

bifida cystica [1]). The vertebrae at the level of the lesion lack neural arches, and so are incomplete dorsally.

Individuals with MMC often exhibit motor and sensory neurological deficit below the level of the lesion. This may result in lower limb weakness or paralysis that hampers or prevents walking, and lack of sensation that enhances the risk of pressure sores. Urinary and fecal incontinence occur frequently, as does hindbrain herniation (Chiari II malformation) and associated hydrocephalus which often requires shunting. Orthopedic abnormalities including talipes (club foot), contractures, hip dislocation, scoliosis and kyphosis are frequently observed. There is a strong correlation between the axial level of lesion and the degree of disability experienced by individuals with MMC. A 40-year follow-up of 117 children whose lesions were repaired in the UK during the 1960s and 1970s found only 17% survivors with lesions above the 11th thoracic vertebra (T11), whereas 61% were alive with lesions below the 3rd lumbar vertebra (L3) [2]. Significantly fewer survivors were community walkers, and were free of pressure sores, in the 'above T11' group compared with the 'below L3' group.

The lifetime cost of a child born with MMC is estimated at over €500,000 ($600,000), of which 37% comprises direct medical costs with the remainder being 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 of spina bifida, considerable effort has been invested in exploring the pathophysiological mechanisms, finding better ways to treat and manage the condition and its consequences, and progressing towards the ultimate goal of primary prevention. This article considers the main areas of progress to date, and looks forward to developments that may further enhance the outlook for people with spina bifida.

I. Epidemiology

Many epidemiological studies lump spina bifida together with the related defect anencephaly, and sometimes also with encephalocele, under the general term 'neural tube defects' (NTDs). Box 1 lists the pathological conditions that are usually considered to be NTDs. Birth prevalence of NTDs has varied considerably over past decades [4] and continues to show substantial differences between geographical locations. For example, the prevalence of NTDs in the USA and many European countries is estimated at 0.5-0.8/1000 births[5] whereas prevalence in some regions of China has been reported to be more than 20 times higher [6]. Assuming an average prevalence of one NTD case per 1000 births, with a global population of 7 billion and birth rate of 20 per 1000 population, this generates a figure of 140,000 NTD cases per year worldwide. Regions of higher NTD prevalence have uniquely shown disproportionately higher frequencies of rarer subtypes such as craniorachischisis and iniencephaly [7]. Further, within-country differences have been observed between racial and ethnic groups. For example, in the USA, Hispanics have higher spina bifida prevalence [8], and African-Americans have lower prevalence [9], compared with non-Hispanic whites. Prevalence differences in time and across geographic regions have been attributed to variations in ascertainment methods as well as to true differences in risk. Indeed, ascertainment of NTDs is challenging based on antenatal screening procedures that can lead to diagnosis and subsequent pregnancy termination. Omission of elective terminations clearly underestimates prevalence and may bias risk estimations in etiologic 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 and stillbirths, generating particularly comprehensive prevalence data for NTDs and other malformations. For the period 2003-2007, EUROCAT estimated the prevalence

(including chromosomally-related disorders) of ‘spina bifida’ and ‘NTDs’ at 0.51 and 0.94 respectively per 1000 births, stillbirths and pregnancy terminations [11].

It has long been known that both genetic and non-genetic factors contribute to NTDs. Heritability (the genetic component of risk) was estimated at 60-70% based on the relative proportions of individuals affected amongst siblings of index cases from prevalence surveys in the 1960s, in South Wales, Glasgow, and London [12]. Fewer than 10% of NTD cases are syndromic, for example occurring in chromosomal disorders including trisomy 13 or 18, while the great majority are non-syndromic and exhibit a sporadic pattern of occurrence. Several lines of evidence support a multi-factorial 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%, therefore representing a 20 to 50-fold increased risk compared with the general population prevalence of ~1 per 1000. Second- and third-degree relatives show lower recurrence risks than first-degree relatives, but still higher than unrelated individuals. For a particular woman, her empirical recurrence risk after an affected pregnancy is approximately 3%, rising to around 10% after two NTD pregnancies. In twins, the concordance for NTDs is higher amongst same-sex twin pairs (monozygotic and dizygotic) than opposite-sex twins (only dizygotic). The finding of a female excess among fetuses/infants with anencephaly, but not with spina bifida, has strongly suggested a sex-related genetic or epigenetic relationship [14]. Finally, the NTD 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 (see Section II).

Considering non-genetic factors, diminished folate status is undoubtedly the best known factor influencing NTD risk (see Section II for further detail). Beyond folate, a number of other nutrients and nutrition-related factors have been linked with NTDs (Table 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 obesity-associated risks are stronger for spina bifida than for anencephaly [16-18] and may not be modified downward with folic acid use [19]. For spina bifida, elevated risks have been consistently observed across studies in the range of 1.5 to 3-fold. In addition, severe obesity (body mass index > 35) has been associated with even larger risks indicative of a "dose-response" relationship linking obesity with spina bifida. Underlying mechanisms that have been suggested 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 including pollutants and personal toxicants (Table 1). However, most of these factors have either not been consistently observed, were relatively infrequent in occurrence, or the associated magnitude in risk for the factor is not very large. Thus, such factors are unlikely to explain a substantive proportion of the population burden of NTDs [20].

II. Mechanisms and pathophysiology

The primary disorder in the pathogenesis of MMC 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 development of spinal motor and sensory function even below the lesion level. As gestation progresses, however, the exposed spinal cord becomes haemorrhagic and neurons die as a result of toxicity of the amniotic fluid (Box 2). Axonal connections are interrupted, and function is lost [21]. Hence, neurological disability

in MMC is often considered a 'two-hit' process: failed neural tube closure followed by neurodegeneration *in utero*. This has encouraged attempts to cover the spina bifida lesion during fetal development, in order to arrest or prevent the neurodegeneration in cases where closure has failed (see Section IV).

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 belong to a wide range of molecular pathways [23] and the mutants display a variety of NTD phenotypes that mimics the range of human NTD variants. Exencephaly, the developmental precursor of anencephaly, is most commonly encountered after gene mutation in mice (over 150 genes), but open spina bifida is also observed in more than 40 mutant strains, and is the only NTD in several cases [22, 24]. Sequencing of the coding regions of human orthologues for many of these genes has revealed rare missense (amino acid-altering) mutations in patients with NTDs, that are absent from unaffected individuals. In particular, variants of genes in the planar cell polarity pathway (PCP), a non-canonical Wnt signalling cascade, have proven to be associated with a variety of NTDs [25]. This is particularly significant, since PCP gene mutations are potent causes of mouse NTDs, generating several phenotypes particularly the severe defect craniorachischisis [26]. A second group of NTD-associated genes are those encoding enzymes of folate one-carbon metabolism (FOCM). Methylene tetrahydrofolate reductase (MTHFR) is an enzyme generating 5-methyltetrahydrofolate, essential for conversion of homocysteine to methionine. Its 677C>T variant, which results in the conversion of valine to alanine at codon 222, reduces the activity of this enzyme and the 677TT genotype, in either mother or fetus, particularly when folate status is low, can be a risk factor for NTDs in populations of non-Latin origin [27]. In mice, knockout of the *Mthfr* gene does not generate NTDs [28], raising a question over the specificity of this genetic association

with NTDs. In contrast, mutations in genes of the glycine cleavage system, which reduce the activity of two mitochondrial enzymes of FOCM (*GLDC* and *AMT*), are also found among NTD patients [29] and in this case loss of function of the mouse orthologues produces NTDs [29] (Pai et al, personal communication). Mitochondrial enzyme activity supplies 70% of the cell's one-carbon units for metabolism, as formate molecules, and it seems possible that genetic variants in this pathway may prove to be important risk factors for NTDs.

Non-genetic factors. Although a variety of environmental factors have been linked with NTDs (Table 1), only a few clues exist to the pathogenic mechanisms. Moreover, it seems likely that non-genetic factors mainly influence neural tube closure when combined with a predisposing genotype. The anticonvulsant valproic acid (VPA) increases risk of NTDs by ~10-fold when taken during the first trimester of pregnancy [30]. Its potent histone deacetylase (HDAC) inhibitory activity may disturb the balance of protein acetylation and deacetylation, leading to neurulation failure [31]. The causation of NTDs by the fungal product fumonisin was demonstrated in studies of an 'outbreak' of NTDs in South Texas, linked to fungal contamination of tortilla flour [32]. The production of NTDs by fumonisin exposure in rodent embryos has identified sphingosine phosphate metabolism as a key target of the toxin, potentially compromising folate utilization [33]. In maternal diabetes mellitus, which predisposes to a range of birth defects including NTDs, hyperglycemia is the immediate cause of NTDs although its pathogenic mechanism is poorly understood. One suggestion is disrupted expression of the *Pax3* gene [34] whose loss of function itself leads to mouse NTDs.

Embryonic pathogenesis of MMC. Two distinct phases of neural tube formation occur in higher vertebrates: primary (closure) and secondary (canalisation). In humans, primary neurulation is initiated at the boundary between future hindbrain and cervical spine on day 22

post-fertilization, from which level the neural tube 'zips up' bi-directionally 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 while 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 [35]. This marks the completion of the spinal cord to the upper sacral level.

NTDs can result from failure of any part of this sequence of neurulation events and are typically open defects, owing to the arrest of closure prior to fusion of the neural folds in the dorsal midline (Figure 1A-C). The most severe spinal defect is craniorachischisis, in which closure fails to be initiated on day 22 in humans, yielding almost completely open brain and spine. Analysis of mice with mutations in PCP genes including *Vangl2* have revealed a defect of late gastrulation. The process of convergent extension involves the intercalation of cells in the midline to lengthen and narrow the body axis but, when this fails in PCP mouse mutants, the body axis remains short and wide. The neural folds are spaced abnormally widely apart and are physically unable to initiate closure [36]. If the embryo successfully initiates closure but fails subsequently in cranial neurulation, then anencephaly results. Failure of subsequent spinal neurulation generates open spina bifida lesions of varying size and axial level, depending on the stage at which the wave of 'zipping' closure arrests. For example, *Zic2* mutant mice fail early in spinal neurulation, owing to lack of dorsolateral neural plate bending [37], and display a large spina bifida from thoracic level downwards. In contrast, spinal closure in the *curly tail* (*Grhl3*) mutant fails later, due to enhanced curvature of the body axis [38], producing a spina bifida confined to the lumbo-sacral region. It is not yet

clear whether human spina bifida of differing axial extents also result from distinct genetic causes, as in mice.

Pathogenesis of closed (skin-covered) spinal dysraphism. Secondary neurulation is responsible for forming the neural tube in the low sacral and coccygeal regions, following closure of the caudal neuropore (Figure 1D). The end of the embryo comprises the tail bud (also called the 'caudal cell mass') whose mesenchymal cell core progressively reorganises into longitudinal cell condensations. The most dorsal of these condensations undergoes 'canalisation', converting the solid neural precursor into a hollow, epithelial secondary neural tube [35, 39]. There is no 'closure' component in secondary neurulation, and so defects ('closed spinal dysraphism') are not open to the external environment, but skin-covered (Figure 1E, F). The principal defect appears to be failure of the neural and mesodermal tissues to become distinctly specified and separated spatially. Recent research has revealed a bipotential neuro-mesodermal precursor cell lineage within the tail bud [40], explaining why this separation is sometimes incomplete. The clinical observation that the distal spinal cord is often 'tethered' to surrounding tissues, in closed spinal dysraphism, can therefore be recognised as a disorder of secondary neurulation. However, the frequent and striking association of closed spinal dysraphism with intradural lipoma (Figure 1F) [41] is not well explained, and is yet to be reproduced in an animal model.

Postnatal pathogenesis. MMC is the main form of spina bifida associated with brain malformations and hydrocephalus. The main brain defects (Figure 2) involve the spectrum of anomalies related to the Chiari II malformation of the hindbrain in about 90% of cases [42]. This is associated with a cerebellum of normal size developing in a small posterior fossa, so that the cerebellum herniates downward through the foramen magnum [43]. Quantitative studies show a reorganization of the cerebellum, in which the anterior part is larger, the posterior-

inferior regions are smaller, and there is no difference in the corpus medullare (cerebellar white matter) [44]. Cerebellar volume reduction is more associated with thoracic level spinal lesions than lumbar or sacral lesions, but both are reduced relative to controls [45]. In addition, about 65% of cases exhibit distortion of the midbrain, often marked by tectal beaking, in which the colliculi fuse into a single 'beak' pointing posteriorly and invaginating into cerebellum. The medulla is elongated and kinked at the spino-medullary junction in about 70% of cases [42].

The basal ganglia and related subcortical structures are visibly normal on radiological review [46]. On quantitative macrostructural assessment, the hippocampus, but not the amygdala, is reduced in volume [47], and the putamen is enlarged. About a third to half of children with MMC have hypogenesis (under-development) of the corpus callosum involving either the splenium and posterior body or the rostrum [42]. These anomalies suggest that the disruption of neural migration associated with MMC is prolonged into the second trimester, since the corpus callosum develops from 8-20 weeks prenatally [48]. Quantitative studies of the corpus callosum show marked volume and integrity differences, especially posteriorly in cases with hypogenesis or severe hypoplasia [49]. Reduced integrity has also been shown in the genu, but not in the anterior commissure [50]. There is recent evidence that corpus callosal defects can also be associated with closed spinal dysraphism [51].

Secondary consequences of MMC include hydrocephalus which results primarily from obstruction of cerebrospinal fluid (CSF) flow at the level of the fourth ventricle, with other contributing factors including aqueductal stenosis, venous hemodynamics and ependymal denudation. Cortical reorganization occurs around the area of ventricular dilatation. On quantitative studies, the frontal regions are enlarged and there is a reduction in the volume of

posterior cortical regions [52]. Hydrocephalus stretches the white matter, which is most apparent in the thinned (hypoplastic) appearance of the corpus callosum [53]. Diffusion tensor imaging of white matter structures shows that the integrity of the long association fiber tracts connecting posterior and anterior brain regions are consistently reduced relative to controls [54, 55]. Using the midbrain as a seed point, Williams et al [56] showed greater reduction in posterior white matter integrity than frontal pathways, especially in association with tectal beaking.

Hydrocephalus exerts primarily a linear effect on cognitive and motor outcomes, reflecting the severity of white matter impairment [57]. Deviations from normative standards for volumes of frontal versus posterior regions are associated with reductions in IQ and fine motor dexterity [58]. The specific contributions of the Chiari II malformation may be under-estimated as factors in cognitive and motoric outcome. Chiari II is associated with eye movement difficulties as well as problems with the precision and timing of motor movements and rhythmicity [59]. Attention deficit is common in MMC, reflecting problems with posterior attention systems involving orienting and arousal mediated by the midbrain, with tectal anomalies directly correlated with the severity of difficulties with stimulus control [60]. In contrast, motor functions such as procedural learning and attention functions involving sustained attention and persistence are relatively preserved, possibly reflecting less impairment in the frontal-striatal regions and basal ganglia [59]. The corpus callosum anomalies are associated with reduced interhemispheric communication and more general difficulties integrating information in language, reading, and social domains [61].

These neurocognitive difficulties can be observed as early as 6 months of age [62], reflecting domain general deficits in timing, attention, and movement that affect the development of people with MMC across the life time. They lead to difficulties in learning to construct and

assimilate information (assembled processing) which contrast with relative strengths in associative and procedural learning (associative processing) that occur within outcome domains (Figure 3) [63].

Intellectual disability is relatively infrequent, affecting perhaps 20-25% of people with MMC and often after complications of hydrocephalus. In US samples, Hispanic individuals have shown a greater frequency of impaired cognitive outcome, which correlates with a specific association of more frequent upper level MMC defects and growing up in poverty [45]. The characteristic cognitive strengths and weaknesses associated with MMC are highly variable and poorly reflected by IQ scores. The strengths reflect preservation of associative processing and include procedural learning, word reading, vocabulary and the form of language, persistence, and social activation. These contrast with weaknesses in motor adaptability, language comprehension and pragmatics, and hypersociality. Sources of variability are the severity of the malformations and hydrocephalus, the treatment of hydrocephalus because of shunt obstruction and infection, and environmental factors involving socioeconomic status [63]. Cognitive and motor outcomes are directly related to spinal lesion level, which reflects the association of more severe brain dysmorphology with higher level defects.

III. Diagnosis, screening and prevention

Biochemical diagnosis and screening. Prenatal diagnosis first became possible in the early 1970s, with the finding of an elevated concentration of alphafetoprotein (AFP) in amniotic fluid samples from pregnancies with anencephaly or MMC [64, 65]. Subsequently, assay of acetylcholinesterase in amniotic fluid was also shown to be diagnostic [66]. While AFP measurement on amniotic fluid samples may be useful for high risk cases, the 1% chance of

miscarriage following amniocentesis limited its more general application. The finding of elevated AFP concentrations in maternal serum samples in MMC [67] greatly enhanced the utility of AFP measurements and formed the basis of subsequent population screening approaches [68] . However, with the increasing use of routine second trimester anomaly scanning, biochemical screening for MMC is becoming redundant as ultrasound offers greater sensitivity and specificity. The main indication for biochemical screening now is maternal obesity where it impairs detailed ultrasound examination of the fetal anatomy.

Sonographic diagnosis. In parallel with the development of AFP diagnosis, the 1970s also saw improvements in ultrasound that led to non-invasive diagnosis of MMC and other NTDs [69]. Today, the fetal spine can be examined by ultrasonography in the sagittal, axial and coronal planes from late first trimester onwards, providing the principal and most accurate mode of prenatal diagnosis. For reliable detection of MMC, 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 MMC, whereas skin-covered (closed) lesions are rarely identified *in utero*. Figure 4 shows views of the normal spine juxtaposed with MMC to demonstrate the sonographic findings. The spinal lesion is most readily identified when examined in the sagittal plane (Figure 4A, B), particularly if associated with a meningocele or MMC when the cystic extension is often visible from the posterior aspect of the spine (Figure 4C, D). The presence of neural tissue within the sac can often be seen, although ultrasound cannot reliably exclude the presence of neural tissue. Varying degrees of distortion of the spine, from virtually none to severe kyphoscoliosis, can also be seen in association with spina bifida.

Several cranial features are associated with spina bifida including a disproportionately small biparietal diameter for gestational age [70] and varying degrees of ventriculomegaly, which may occur in almost all fetuses by the third trimester but is present in up to 70% of cases in the second trimester [71]. In the late 1980s, the 'lemon' and 'banana' signs (Figure 4E-H) were described [72]. These cranial signs have been a significant aid to prenatal diagnosis, since the head is examined routinely in all fetuses in the second trimester, whereas detailed spinal examination may be compromised by fetal position or other technical factors such as maternal habitus. However, recognition of the cranial signs should be an indication to ensure that detailed examination of the spine is undertaken and, in many units, may result in tertiary referral. Subsequent to the recognition of these cranial signs, routine second trimester ultrasound now detects around 90-98% of fetuses with MMC in countries offering routine second trimester anomaly scanning (Table 2)[73]. Whilst studies reporting detection rates using routine ultrasound scanning are now more than ten years old, and obesity is increasingly common in the obstetric population, ultrasound technology has improved significantly and there is no doubt that routine fetal anomaly scanning will continue to have a significant impact on the prenatal detection of neural tube defects, as previously [74]. In the UK National Ultrasound Screening Programme the minimum standard for the detection of this anomaly following routine second trimester anomaly scanning is 90% (UK National Screening Committee; http://www.fetalanomaly.screening.nhs.uk/standards).

The lemon sign refers to a loss of the convex outward shape of the frontal bones with mild flattening (Figure 4E, F), and is present in virtually all fetuses with MMC between 16 and 24 weeks' gestation. It is less reliable after 24 weeks, when present in only 30–50% of cases (Table 3)[75-78]. The banana sign refers to the shape of the cerebellum (Figure 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 [79]. Cerebellar abnormalities are present in 95% of fetuses irrespective of gestation. However, the cerebellar abnormality seen most commonly before 24 weeks' gestation is the banana sign (72%) whereas in later pregnancy the cerebellum is more often absent from view (81%) (Table 3) [80].

Following the identification of spina bifida, detailed examination of the fetus is performed to look for other signs which may 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 may suggest an associated chromosomal abnormality (e.g. advanced maternal age) [78, 81]. Prediction of spinal level of the lesion, with its prognostic significance, would be advantageous, and one study using 3-D ultrasound has reported an accurate sonographic estimation of the defect level to within one spinal segment in 86% of cases [82]. However, anatomical level may not correspond to functional level, and ultrasound was not found to be predictive for postnatal mobility or intellectual function [83].

Prevention. The prevention of NTDs by folic acid has been heralded as a modern public health success [84]. Nearly 40 years ago, Smithells and colleagues found that diets and postpartum blood levels of women who had a pregnancy affected by NTD were mildly deficient for selected micronutrients, including folate [85]. A folate-containing multi-vitamin supplement reduced the risk of NTD recurrence in women with a previously affected pregnancy [86]. Subsequently, the MRC randomized clinical trial of NTD recurrence [87], a randomised trial of NTD first occurrence [88], and a number of observational epidemiological studies, all provided evidence that folic acid supplements can prevent many NTD-affected pregnancies. Now, 'high risk' women, with a previous history of a NTD-affected pregnancy,

are recommended to take 4 mg folic acid while planning a pregnancy, whereas those at low risk are advised to take 0.4 mg [84].

Concerns about the effectiveness of voluntary folate supplementation have led to folic acid fortification of staple foods and other policy promotions of folic acid in many countries. Mandatory folic acid fortification of cereal grain products in the USA began in January 1998, and has been associated with a reduction in prevalence of NTDs of approximately 25% [89]. Implementation of mandatory fortification programs elsewhere, as in Chile [90], Costa Rica [91], Canada [92], South Africa [93] and Saudi Arabia [94] has been associated with similar or even greater reductions (e.g. >50%) in NTD prevalence, particularly spina bifida, whereas Brazil [95] and Peru [96] did not report a reduced NTD prevalence after fortification programmes. The relative amount of reduction in prevalence appears roughly correlated with the magnitude of the initial prevalence of NTDs. Some countries have also observed reductions in NTD prevalence after implementing programs of voluntary folic acid supplement use or fortification [97]. Considerable discussion remains around establishing national mandatory fortification programs in other countries, for example throughout Europe. Some scientists have questioned whether these programs have gone far enough in reaching susceptible pregnancies [98] while others have expressed the need to balance the benefits of NTD prevention with possible risks for other parts of the population [99, 100].

The underlying mechanisms by which folic acid facilitates NTD risk reduction remain unexplained [84]. Also unknown is why a substantial proportion of women who take folic acid supplements in the periconceptional period still experience NTD-affected pregancies. Recent investigations have explored genetic variation in folate transport and metabolism [101-103] and the role of autoantibodies against the folate receptor, which are a possible cause of maternal

immunological response hampering folate uptake [104]. The small molecule inositol which is essential for a number of intracellular signaling pathways and is a building block for membrane phospholipids, can prevent mouse NTDs that do not respond to folic acid [105]. Encouraging preliminary results with inositol supplements have emerged in humans [106], but need to be verified by clinical trial.

IV. Management

The management of MMC traditionally involves surgery within 48 h of birth. The child's back is closed to minimize the risk of ascending infection that can 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. Neonates with spina bifida 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. Virtually all neonates with thoracic level lesions need a ventriculo-peritoneal shunt, whereas around 85% of patients with a lumbar level lesion, and about 70% with a sacral lesion, require shunting [107]. Over the last five years, endoscopic third ventriculostomy with choroid plexus coagulation has become an alternative treatment for hydrocephalus associated with spina bifida, in highly selected cases [108]. Radiologic evidence of the Chiari II malformation is present in most individuals, and clinically symptomatic hindbrain herniation may affect up to 30% of cases. This manifests as apnea, swallowing difficulties, and stridor in a newborn baby, or headache, quadriparesis, scoliosis, and balance/coordination issues

in an older child. In severe cases, posterior fossa decompression surgery is indicated [109].

Orthopedic deformities are usually treated shortly after birth, with long-term follow-up. Patients are also monitored by ultrasonography and urodynamic studies to detect urological complications resulting from abnormal neurological bladder function. These 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 includes a combination of clean intermittent catheterization, pharmacological agents, and surgery [110]. Bowel function is not an issue in neonates, but older children require bowel management including the use of suppositories, enemas or laxatives [111], and the use of antegrade colonic enemas [112].

Medical management of individuals with spina bifida is best provided through regular assessments by a multidisciplinary team, directed by a physician with training in the care of children with spina bifida, and including a coordinator with responsibility for patient follow-up. Additional team members include a nurse specializing in the care of children with multiple handicaps, a pediatric neurosurgeon, urologist, and orthopedic surgeon, a physical therapist, and a social worker. Other subspecialists, for example a psychologist, may become involved if required in individual cases. Communication is vital between the multidisciplinary team members, and with the patient's primary physician, who provides routine medical care including immunizations and continuing emotional support for the family. Additional issues that may need to be addressed by the team include neurobehavioral development (see

Section V), mobility and means of locomotion, weight maintenance, skin care, and the avoidance of latex sensitization.

Fetal surgery. The rationale for fetal surgery [113] is that damage to the exposed spinal cord is progressive during gestation (see Box 2 and Section II): hence early repair of the lesion, *in utero*, may prevent continuing damage and improve clinical outcome. Additionally, spina bifida repair arrests the leak of CSF from the lesion, enabling reversal or resolution of hindbrain herniation [114-116].

Pregnant mothers with a diagnosis of MMC, who consider *in utero* surgery, undergo extensive prenatal testing. This includes: obstetric evaluation; screening for genetic or chromosomal syndromes (see Section III); ultrasonography to assess lower extremity function, identify club foot anomalies and estimate the spinal level of the defect by localizing vertebral arch defects; fetal echocardiography; and ultrafast MRI to assess the presence or absence of hindbrain herniation, hydrocephalus, and any other brain abnormalities [117].

The intraoperative and postoperative management algorithm for fetal MMC surgery [118] involves maternal laparotomy followed by hysterotomy using a uterine stapling device, after which the fetus is positioned with the spinal lesion visible through the uterine wound (Figure 5). The fetal heart is monitored by intraoperative echocardiography [119]. The cystic membrane of the MMC is excised and the attachments of the meninges to the skin and soft tissues are freed. If possible, native dura is closed over the neural placode as a first layer, followed by creation and midline closure of paraspinal myofascial flaps. Skin flaps are widely mobilized and

closed to complete the repair although, when the skin cannot be closed primarily, an acellular human dermis graft is used to complete the closure.

Successful *in utero* spina bifida repair was first reported in 1998 [120, 121], and clinical experience grew rapidly thereafter, with promising results [114, 115]. In 2003, as the fetal surgery approach was becoming increasingly widespread, but without compelling proof of safety or efficacy, a prospective randomized clinical trial was initiated. The objective of the National Institutes of Health (NIH)-supported Management of Myelomeningocele Study (MOMS) was to evaluate whether intrauterine repair of MMC between 19 and 25 weeks gestation improved outcomes compared with standard, postnatal neurosurgical repair [118]. Standardization of patient inclusion and exclusion criteria, and all prenatal and postnatal patient care protocols, was established at the three participating clinical centers: The Children's Hospital of Philadelphia, Vanderbilt University, and the University of California, San Francisco. MOMS involved two primary outcomes: first, a composite of fetal or neonatal death or the need for ventriculoperitoneal shunt placement by the age of 12 months and, second, an assessment of mental development and motor function at 30 months of age. A variety of secondary neonatal and maternal outcome measures were also examined such as complications of premature birth. During the study, the investigators were blinded to the results: follow-up evaluation of the children and mothers was performed by an independent medical team of pediatricians and psychologists appointed and supervised by the Data Study and Coordinating Center at George Washington University.

In December 2010, enrollment was stopped by the Data Safety and Monitoring Board because of the efficacy of fetal surgery after recruitment and randomization of 183 of a planned sample of 200 patients. Confirming the earlier, non-randomized results of patients who underwent fetal MMC repair, the MOMS trial showed a significant reduction of ventriculoperitoneal shunt placement at one year of age following fetal surgery (prenatal group: 40%; postnatal group: 82%). The trial also demonstrated an improvement in overall neuromotor function at 30 months of age by a variety of measures including the finding that 42% in the fetal surgery group were walking independently compared with only 21% in the postnatal surgery group. This was despite the fact that on average, the prenatal surgery group contained higher and more severe MMC lesions than the postnatal group. Hindbrain herniation was also significantly reversed in the fetal surgery group compared with the postnatal surgery group. On the negative side, fetal surgery was associated with significant risks related to premature birth (average gestational age at delivery in the fetal surgery group was 34.1 weeks gestation compared with 37.3 weeks in the postnatal surgery group). Moreover, about 25% of mothers in the fetal surgery group demonstrated evidence of thinning of the uterine wound at the time of cesarean delivery, and 10% showed partial (9%) or complete (1%) tissue edge separation at the hysterotomy site, although none had a hysterotomy rupture.

A mother carrying a fetus with MMC, at less than 24 weeks gestation now has three choices: termination of pregnancy, continuation of the pregnancy with near-term cesarean section and postnatal repair, or prenatal surgery if she satisfies the criteria for this procedure (Box 3). A study that used MOMS data and a financial model showed health care savings of $2,066,778 for every 100 cases of fetal spina bifida repair

performed [122]. Long-term follow-up is crucial to assess the durability of the initial benefits, and the NIH has funded a study of the MOMS trial patients at 6-10 years of age. The clinical experience with fetal MMC repair during the past 3 years, since the MOMS trial, has shown comparable results [123]. Institutional guidelines have been established for fetal MMC repair and, for patient safety and optimal outcome, fetal MMC surgery should be limited to high-volume fetal surgery centers with a committed multidisciplinary team of experts following a standardized patient care protocol [124]. A data registry to collate the outcomes for patients who undergo fetal MMC repair has been established by the North American Fetal Therapy Network (www.NAFTNet.org).

V. Quality of Life

MMC has a pervasive impact on the physical, neurocognitive, psychological and social functioning of affected individuals [125-127].

Health-related quality of life (HRQOL). Children and adolescents with spina bifida have a reduced HRQOL compared with individuals without spina bifida and those with other chronic health conditions. These differences tend to be stable across age groups, sex, geographical location and time [128, 129]. Although measures of spina bifida severity such as lesion level, continence status and outcomes of various surgical procedures tend not to be associated with HRQOL [129, 130], other factors are significantly associated, particularly the presence of shunted hydrocephalus and lack of mobility [131, 132]. Other robust predictors of HRQOL effects include social class, pain levels, parenting stress, and other family factors [129, 133].

Psychosocial adjustment. During late childhood, people with spina bifida tend to exhibit higher levels of depressive symptoms and lower levels of self-concept than unaffected individuals [134-136]. Children with spina bifida also exhibit social difficulties: they tend to be socially immature and passive, have fewer friends, be less likely to have social contacts outside of school, and to date less during adolescence [134, 137, 138]. Most of these difficulties appear to be maintained into young adulthood [135]. During childhood and adolescence, individuals with spina bifida also tend to be more dependent on adults for guidance, less likely to exhibit behavioral autonomy at home and intrinsic motivation at school, and less likely to express their own viewpoints during observed family interactions [134, 139-141].

Family functioning. Research on families of children and adolescents with spina bifida [142] support a resilience-disruption view of family functioning [143]. Although the presence of a child with spina bifida may disrupt normative family functioning, many families nevertheless adapt to such disruption and exhibit considerable resilience, exhibiting levels of family conflict similar to those with typically developing children. Between 10% and 15% of families containing children with spina bifida exhibit clinical levels of 'family dysfunction' [144, 145], but these rates are lower than the 35% dysfunctionality found amongst families of children with cerebral palsy [145]. Families of children and adolescents with spina bifida from backgrounds of lower socioeconomic status are at particular risk for lower levels of family cohesion, supporting a cumulative risk view of such families [146].

Although findings are mixed with respect to marital functioning among parents of children with spina bifida [147-149], the quality of the marital relationship prior to the

birth of the affected child is an important predictor of subsequent family adjustment. A meta-analysis of 15 studies [150] found medium to large negative effects for the impact of spina bifida on parents' psychological adjustment, with somewhat larger effect sizes for mothers (d = 0.73) than for fathers (d = 0.54), as well as negative effects on parental stress levels and parenting quality [141, 151]. Such parents feel less satisfied and competent as parents, feel more isolated, are less adaptable to change, and hold less optimistic views about the future than comparison parents [148, 152, 153]. Parents who are single, older, socially isolated, from an ethnic minority or a low socioeconomic background are at particularly high risk of such outcomes [146, 154]. Siblings of children with spina bifida are better adjusted when they are from families with more positive attitudes toward spina bifida, greater family satisfaction, and lower levels of sibling conflict [155].

Adult outcomes. The mortality rate among young people with spina bifida is roughly 1% per year from age 5 to 30, with the rate being highest among those with the highest level lesions [156, 157]. Among survivors, the quality of individuals' health tends to decline from adolescence to young adulthood, presumably due to difficulties in navigating the transition to adult health care [158-160]. Regarding psychosocial adjustment, emerging adults with spina bifida, like their younger counterparts, are at risk of depressive symptoms and anxiety [132, 161], but they are less likely to engage in risky behaviors such as alcohol use and multiple sexual partners, possibly due to their lower rates of social integration [162]. Regarding educational and vocational outcomes, 41-56% of young adults with spina bifida go to college compared with 66% of typically developing young people [131, 156, 163, 164]. Moreover, recent studies report that only 36-48% of individuals with spina bifida are in full- or part-time employment [131,

[159, 164-166], significantly lower than the rates in typically developing young people (e.g. 75%; [163, 164, 167]) and in those with other chronic conditions (e.g. 68-78% in asthma or cancer; [159, 168]). Moreover, half of those with spina bifida who work have part-time positions and, thus, their annual salary is below the national average [131].

With respect to relationship quality, 43-77% of individuals with spina bifida live with their parents [131, 156]. Half (52-68%) have had a romantic relationship [131], although this rate is lower than in typically developing young adults [164, 169]. The lowest level of life satisfaction is in the areas of romantic relationships, employment, and financial independence [131]. Parents of young people with spina bifida are less likely to discuss issues of sexuality with their offspring [137, 170], and most affected individuals had an inadequate level of knowledge in this area [171]. The high rate of obesity in this population (i.e., rates tend to be over 40%; [172]), coupled with their continence issues, likely undermine young adults' efforts to have romantic relationships [172, 173]. Moreover, participation in leisure and recreational activities tends to be low, with over 50% failing to participate at all [174]. The commonest barriers are lack of motivation, lack of information, and time constraints [174]. Younger individuals and those without shunts tend to participate more than older and more impaired individuals [175].

More generally, the best predictors of successful navigation of young adult milestones appear to be condition-related (i.e. absence of hydrocephalus and good mobility [131]), neuropsychological (e.g. executive functioning [164]), personality-based (e.g. intrinsic motivation [164]), familial (e.g. socioeconomic status, parental intrusiveness [164]), and logistical (e.g. transportation, accessibility [176]). Other factors include financial concerns including lack of health insurance [177], lack of job training and vocational

rehabilitation services, employment discrimination, stigmas related to physical appearance, and a lack of autonomy-related socialization during early childhood [173, 178, 179].

V. Outlook

Spina bifida impacts 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 in the coming years, both enhancing our ability to promote primary prevention, and improving the lives of individuals who have spina bifida.

Genetic basis. Unravelling the causation of spina bifida, like many other diseases, 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 [180] but, even if causal genetic variants are identified in individuals or families with spina bifida, many are likely to be 'private' and not relevant to spina bifida causation in general. Moreover, genetic risk may be imparted by non-coding DNA variants (e.g. enhancer polymorphisms) and specific epigenetic signatures [181, 182], neither of which is detected by exome sequencing. What is needed is a more broad-based approach to the unbiased identification of genomic or epigenomic alterations within groups of individuals with spina bifida (and other NTDs) compared with unaffected controls. An international collaborative approach will be required for this type of study [182]. With continued cost reductions and increase in speed of high throughput technologies, the application of more comprehensive and integrated 'omics' methodologies, including protein and metabolite detection and quantitation, seem likely to be implemented in the coming years [183].

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. Exogenous folic acid may enhance embryonic cell proliferation through stimulation of pyrimidine and purine synthesis, and the finding of disordered embryonic cell proliferation in several mouse NTD models [184] supports such a role. In addition, folic acid may enhance the methylation of key macromolecules including DNA, which can affect embryonic gene expression, thereby contributing to the epigenetic regulation of early nervous system development [181, 182]. A further possibility is that folic acid could in some cases be detrimental for neural tube closure, worsening fetal outcome and leading to miscarriage [185]. Such a detrimental effect, termed 'terathanasia', could in principle account for a reduced NTD prevalence in later pregnancy. Indeed, multi-generational treatment with high dose folic acid increased the frequency of NTDs in two genetic mouse strains [186], whereas NTD frequency in another strain was reduced by folic acid administration and increased by dietary folate deficiency [187], consistent with true primary prevention. Once we are able to identify specific sub-groups of human spina bifida, for example from their genetic risk factors, then it may be possible to determine whether folic acid supplementation interacts heterogeneously with spina bifida in humans, as in mice.

Primary prevention. Finding ways to prevent more cases of spina bifida is a priority for future research and public health implementation. Folic acid food fortification will be extended to countries where this is not currently practised [98]. Moreover, several adjunct or alternative supplementation strategies are under active consideration. Supplements containing vitamin B12, a co-factor in folate one-carbon metabolism, may further reduce the frequency of NTDs [188]. In addition, some NTDs may fail to respond to exogenous folic acid

owing to defects in the intervening metabolic enzymes required to transfer one-carbon units to key downstream metabolites. In this case supplementation with alternative folates, such as 5-methyl tetrahydrofolate [189], or with key downstream molecules such as nucleotide precursors [190], may enhance primary prevention. Apparent 'folate non-responsiveness' is increasingly observed, as women continue to experience spina bifida-affected pregnancies despite taking folic acid supplements. This likely reflects an embryonic defect that cannot be 'corrected' by altering folate one-carbon metabolism, and quite different preventive strategies may be required. Prominent amongst these is the use of inositol, which can prevent NTDs in a mouse strain that is folate non-responsive [105] and has proven well tolerated and associated with normal fetal outcomes in a group of women at high risk of spina bifida [106]. A pilot randomized clinical trial of inositol, alongside folic acid supplementation, is underway in the UK.

Fetal surgery with stem cells. Building on the success of the MOMs clinical trial (Section III), studies are beginning to evaluate the effect of introducing stem cells into the open spinal cord at operation. If stem cells are able to differentiate into neurons or glia, to replace damaged cells or those that have died within the lesion, then neurological function might be enhanced after birth. To date, these studies have transplanted stem cells into rat fetuses with MMC, induced by maternal administration of retinoic acid, and into sheep fetuses with artificially created MMC. Mesenchymal stem cells (MSCs), neural stem cells (NSCs) and skin-derived induced pluripotent stem cells (iPSCs) treated to enhance neural crest cell differentiation, were all shown to survive for variable periods after transplantation [191-193]. Importantly, biodegradable tissue scaffolds have been successfully inserted at fetal operation, enabling cells to be seeded and established on the scaffold prior to transplant [193]. On the other hand, the goal of using autologous (i.e. host-derived) amniotic fluid stem cells (AFSCs) for

transplantation, in order to minimise the risk of graft rejection, may prove problematic as AFSCs from human fetuses with spina bifida fail to deposit collagen type I, and show reduced collagen-related gene expression compared with AFSCs from normal fetuses [194]. A further stem cell-related advance has been the demonstration that autologous bone marrow-derived stem cells can be used, together with a tissue scaffold, to enable bladder tissue engineering, as a possible replacement for the surgical procedure of enterocystoplasty, in which bowel wall is used to reconstruct the bladder: a procedure commonly performed in children with MMC. As with all new stem cell-related therapies, a great deal of work will be needed, both *in vitro* and in animal models, to develop optimum protocols for both efficacy and safety, before stem cell transplants can be considered in human fetuses. Furthermore, to maximise effectiveness of any potential in-utero treatment early sonographic diagnosis will be needed, and will require development of routine sonographic screening programmes delivered around 12 weeks gestation.

Psychosocial developments and interventions. Several domains in the lives of individuals with spina bifida need further research. This is exemplified by the current lack of family-based interventions for families of young people with spina bifida [142], in contrast to the extensive literature in this area for other chronic physical conditions (e.g. type 1 diabetes). Although few randomized clinical trials (RCTs) have been reported in any of the salient psychosocial domains (e.g. quality of life, social skills, independent decision making, depressive symptoms), a recent study found that goals management training reduced anxiety and psychological distress in a small randomized study of adults with spina bifida [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 (i.e. parent and camper) goal identification,

group sessions consisting of psycho-education and the acquisition of cognitive tools, and goal monitoring by camp counselors. Goals for each camper included a medically-related goal (e.g. catheterizing independently) and a social goal (e.g. making a new friend during camp). Statistically significant gains occurred in individualized goals and in the independent management of spina bifida-related responsibilities, with medium effect sizes [196]. Such gains were maintained at 1 month follow-up, and the findings have been replicated with larger effect sizes [197]. Progress towards objective evaluation of such interventions could significantly improve the lives of individuals with spina bifida.

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Figure legends

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.

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.

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).

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**).

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**).

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.

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.

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

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.

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

Text boxes

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]

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].

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.

Box 4. Inclusion and exclusion criteria for fetal repair of myelomeningocele

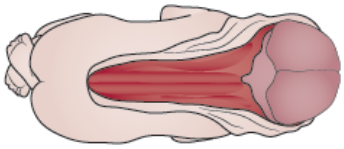
Inclusion criteria

- 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

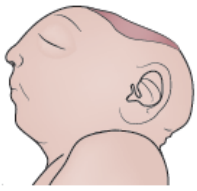
Exclusion criteria

- 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].

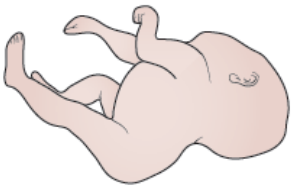


Craniorachischisis
Completely open brain and spinal cord

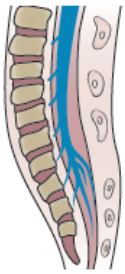


Anencephaly
Open brain and lack of skull vault

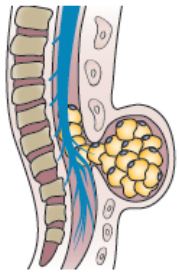
Encephalocele
Herniation of the meninges (and brain)



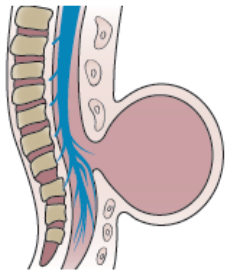
Iniencephaly
Occipital skull and spine defects with extreme retroflexion of the head



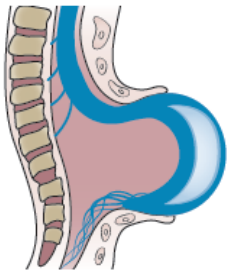
Spina bifida occulta
Closed asymptomatic NTD in which some of the vertebrae are not completely closed



Closed spinal dysraphism
Deficiency of at least two vertebral arches, here covered with a lipoma

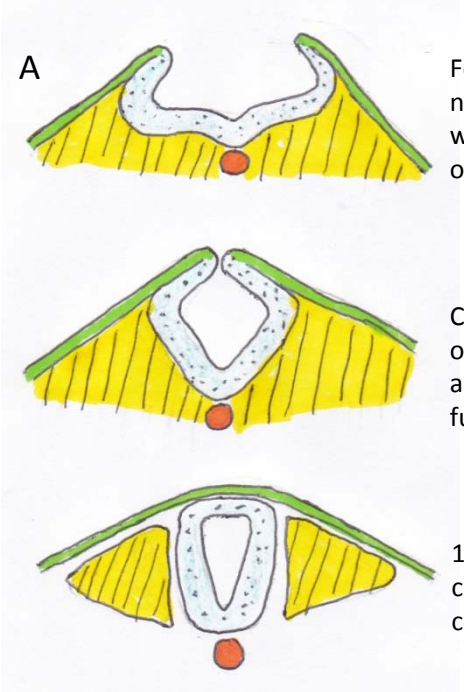


Meningocele
Protrusion of the meninges (filled with CSF) through a defect in the skull or spine



Myelomeningocele
Open spinal cord (with a meningeal cyst)

Primary neurulation, days 22 - 26



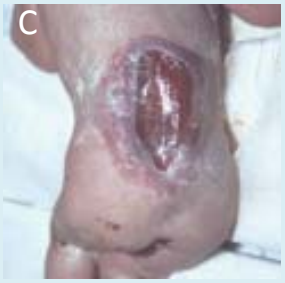
Formation of neural groove with bending of neural plate

Convergence of neural folds and start of fusion

1º neural tube closure complete

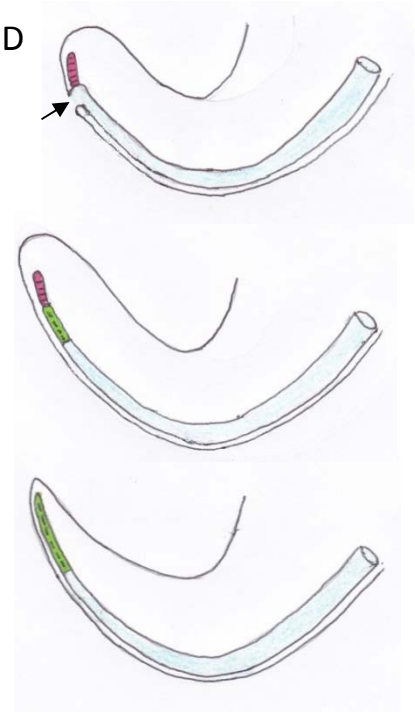
Primary neurulation

Closing neural groove



Open spina bifida after failed closure

Secondary neurulation, days 20 - 40

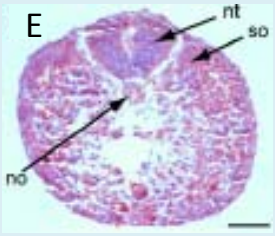


1º neural tube (blue) completed at the caudal neuropore (arrow); medullary cord (red) appears

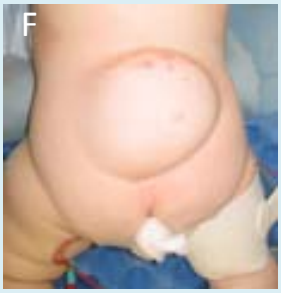
First formation of 2º neural tube (green) by canalisation of medullary cord

Completion of 2º neurulation prior to regression of the tail

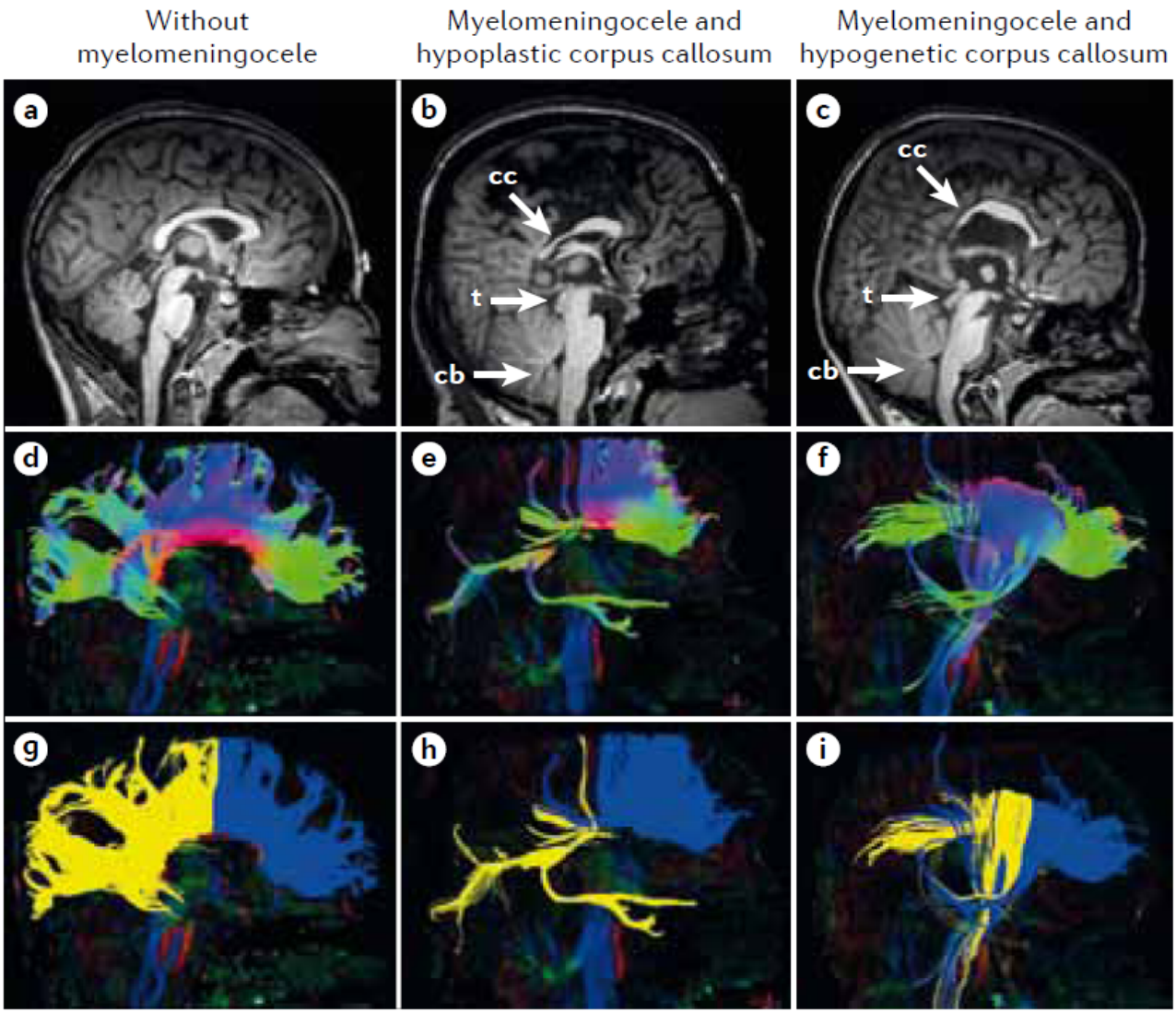
Secondary neurulation



Formation of 2º neural tube by canalisation



Skin-covered spinal dysraphism with lipoma


Without myelomeningocele
Myelomeningocele and hypoplastic corpus callosum
Myelomeningocele and hypogenetic corpus callosum
a
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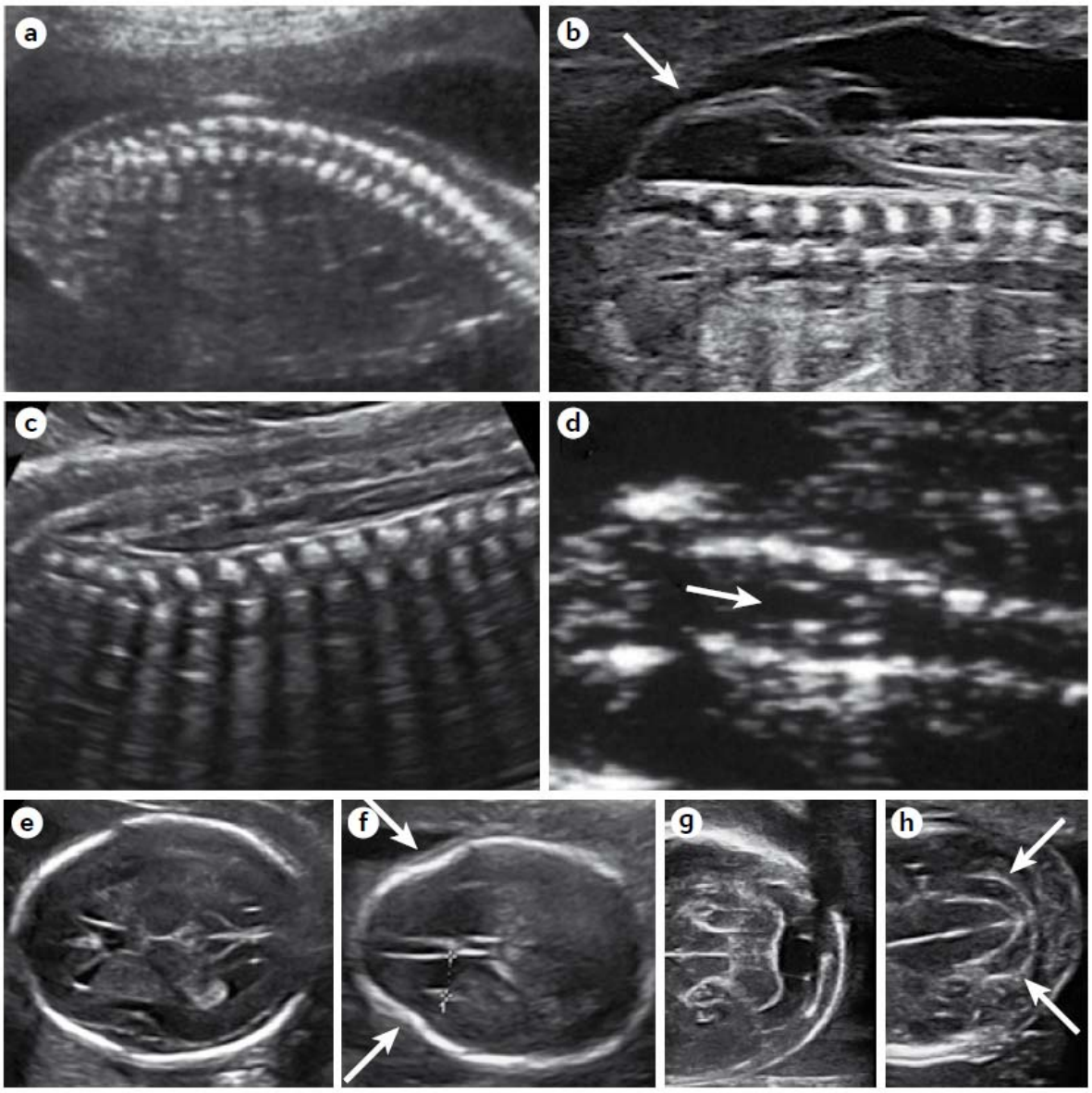
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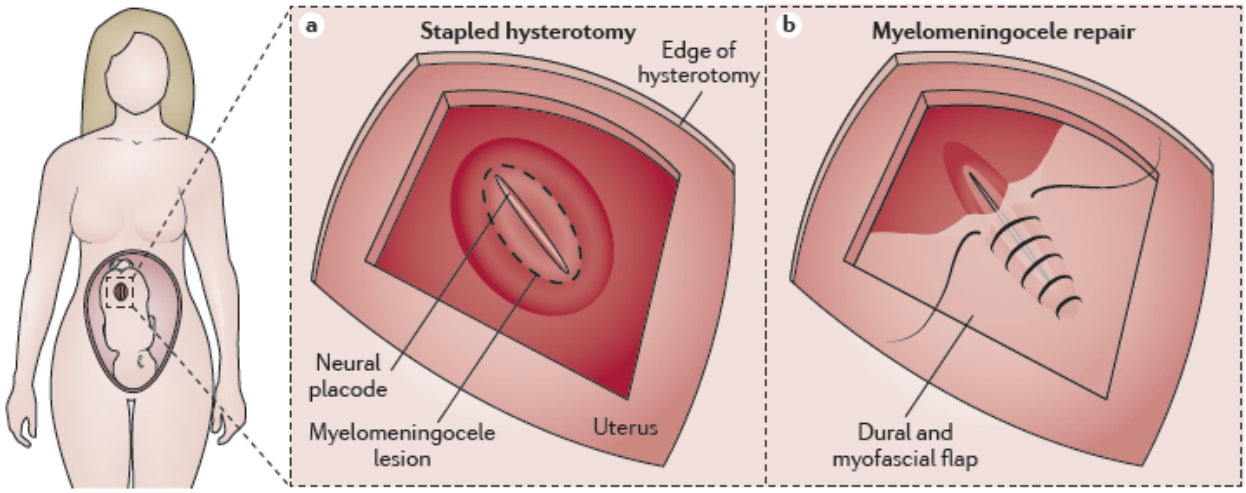
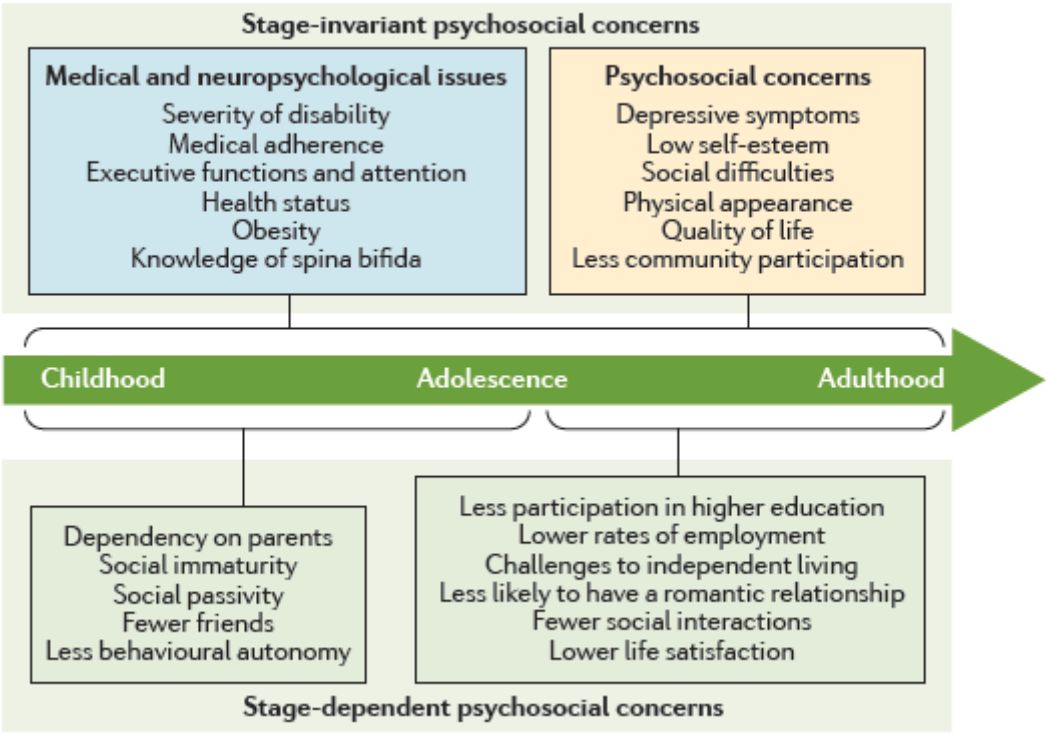
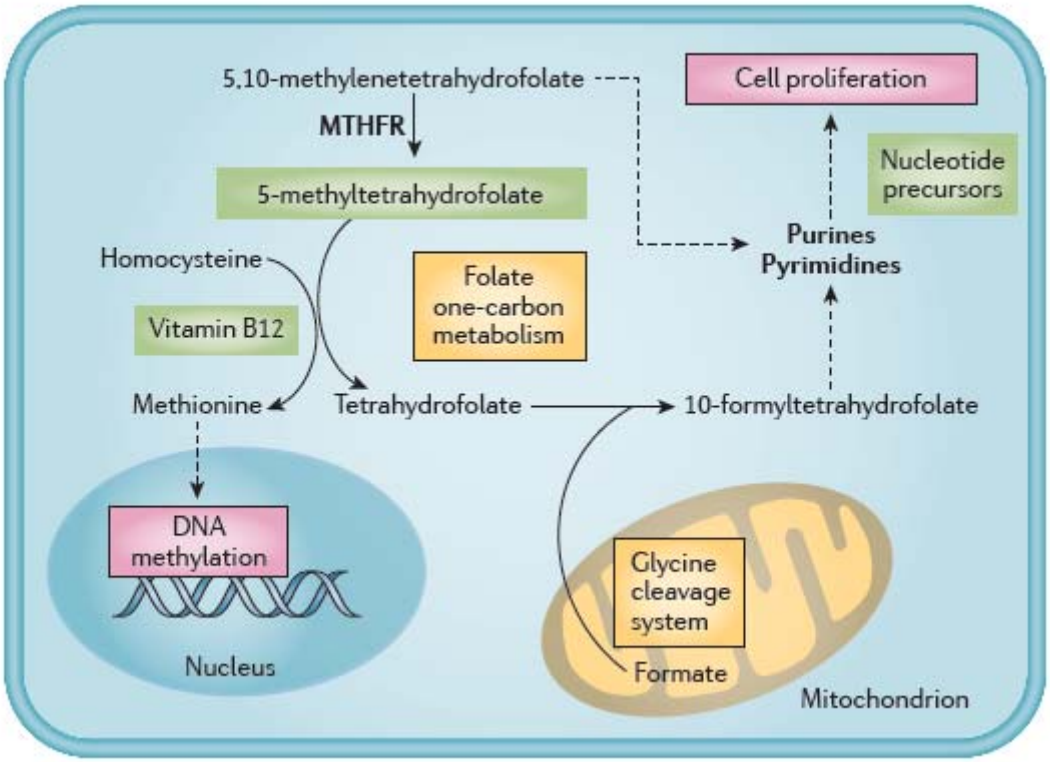

a
Stapled hysterotomy
Edge of hysterotomy
Neural placode
Myelomeningocele lesion
Uterus
b
Myelomeningocele repair
Dural and myofascial flap


Stage-invariant psychosocial concerns
Medical and neuropsychological issues
Severity of disability
Medical adherence
Executive functions and attention
Health status
Obesity
Knowledge of spina bifida
Psychosocial concerns
Depressive symptoms
Low self-esteem
Social difficulties
Physical appearance
Quality of life
Less community participation
Childhood
Adolescence
Adulthood
Dependency on parents
Social immaturity
Social passivity
Fewer friends
Less behavioural autonomy
Less participation in higher education
Lower rates of employment
Challenges to independent living
Less likely to have a romantic relationship
Fewer social interactions
Lower life satisfaction
Stage-dependent psychosocial concerns


5,10-methylenetetrahydrofolate
MTHFR
5-methyltetrahydrofolate
Homocysteine
Vitamin B12
Methionine
Folate one-carbon metabolism
Tetrahydrofolate
10-formyltetrahydrofolate
Cell proliferation
Nucleotide precursors
Purines
Pyrimidines
DNA methylation
Nucleus
Glycine cleavage system
Formate
Mitochondrion