CHAPTER 2

Embryology of Split Cord Malformations

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Table of Contents

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Introduction

Split cord malformation (SCM) defines the group of disorders where the spinal cord is split into two by either a bony or fibrous septum. Based on the nature of the dividing structure, it has been classified into types I and II. However, there have been several reports in the literature where complex multilevel splits are described—some of them in association with other congenital disorders. The pathogenesis of complex SCM has always been intriguing and there is still an ongoing search for the explanation of these presentations based on embryology. Authors intend to go through the natural embryological process in this chapter and also analyze the proposed theories of embryogenesis as described in literature.

Normal Early Human Embryo Development (Journey from 2-Layered to 3-Layered Structure)

■ Gastrulation

In the postovulatory days (POD) 1 to 13, which is the first 2 weeks postfertilization, the human embryo cells divide and undergo rearrangements. It results in the formation of a blastocyst, a two-layered embryo suspended between the amniotic and yolk sacs. This is followed by formation of epiblasts on the dorsal surface of embryo and hypoblast on the ventral surface by postovulatory day 4. Prochordal plate is formed by the thickening of the cranial end of the embryo by postovulatory day 13.

This is also the time when primitive streak develops at the caudal end of the embryo and progresses cranially over the next 3 days. By day 16, it has obtained its full length. It is in the midline in the caudal half. The regression of the primitive streak begins thereafter, and it moves back toward the caudal pole of the embryo. Meanwhile, epiblasts migrate toward the primitive streak through the primitive groove running along the length of the primitive streak. Future endodermal cells ingress and displace the ventrally placed hypoblast cells laterally and form the endoderm. With the regression of the streak, future mesodermal cells ingress between the epiblast and endoderm to form the definitive mesoderm. The epiblast cells that are remaining now spread out and replace the ingressed cells to form both the neuroectoderm and surface ectoderm. It should thus be remembered that the embryonic endo-, meso-, and ectoderm are all derivatives of the epiblast.

The Hensen node needs special mention. Located at the cranial end of the primitive streak, this node acts as the organizer of the embryo. It is through this node that the future endodermal cells migrate, as the streak is elongating, and the future notochordal cells are laid down in the midline between the neuroectoderm and endoderm as the notochordal process.

Prospective neuroectoderm have been localized on the epiblast area surrounding the Hensen node toward the rostral half of the primitive streak. Neuroepithelium can be divided into areas that contribute to multiple neuraxial levels.

Timeline

- POD 4: Formation of epiblast and hypoblast.
- POD 13: Prochordal plate formed at the cranial end.
- POD 16: Primitive streak obtains full length at caudal end.
- POD 23-25: True notochord is formed.
- POD 24-25: Cranial neuropore closes.
- POD 25-27: Caudal neuropore closes.

Notochord Formation

This process starts postovulatory day 16 onward. Cord of cells arranged radially around the notochordal canal constitutes notochordal process. This notochordal canal is in continuity with the amniotic cavity dorsally through the primitive pit. It is between postovulatory days 17 and 21 that the notochordal process elongates. Fusion with the underlying endoderm happens between postovulatory days 18 to 20 and results in the formation of notochordal plate. The notochordal plate gets incorporated into the yolk sac roof and establishes the continuity of notochordal canal. On postovulatory days 17 to 19, neurenteric canal is formed (**Fig. 2.1**). The "true notochord" is formed by postovulatory days 23 to 25, when the plate folds dorsoventrally and separates from the endoderm. This results in obliteration of the neurenteric canal, and continuity with the amniotic and yolk sacs is closed.

Formation of the Neural Tube

By postovulatory day 16, the neuroectoderm is present in the form of pseudostratified columnar epithelium. This overlies the midline notochord and continues laterally, transitioning into squamous epithelium of the cutaneous ectoderm. Neural groove is now visible as a shallow midline fold above the midline notochord on postovulatory days 17 to 19. This groove deepens and forms the neural folds laterally by postovulatory days 19 to 21. These neural folds then elevate and converge toward the midline. They meet to form a closed neural tube. The cutaneous ectoderm apposes and fuses first, followed by neuroectoderm. The disjunction involving the separation of neuroectoderm from cutaneous ectoderm then follows.

Neural tube closure happens over a 4 to 6-day period. Caudal rhombencephalon or cranial spinal cord closes first. Now, it is accepted that this closure happens in several waveforms along the craniocaudal axis rather than in a linear manner like a zipper. The cranial neuropore closes on postovulatory days 24 to 25 in a coordinated fashion with at least four waves interacting. On postovulatory days 25 to 27, caudal neuropore closes. By this time, there are nearly 25 somites formed. Just below the last visible somite, the caudal neuropore is located. The site of closure can be assessed by calculating the distance between the last visible somite and the caudal neuropore. By this calculation, it can be assumed that the

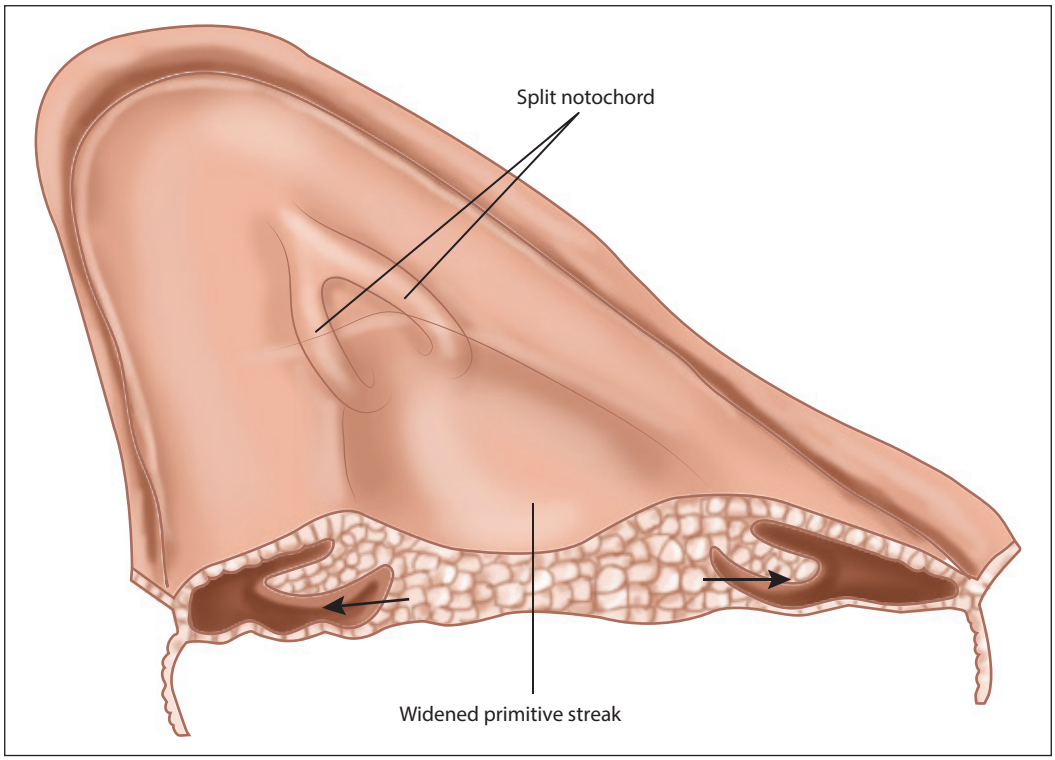

Fig. 2.1 View from above onto the dorsal surface. The caudal pole of the embryo is oriented toward 11 o'clock. During gastrulation, if the primitive streak is abnormally wide, prospective notochordal cells in Hensen's node may begin ingressing more laterally than usual. The paired notochordal streams would not integrate into a single midline notochord and would remain instead as paired paramedian notochordal processes. The caudal neuroepithelium induced by the paired notochordal processes would fail to integrate into a single midline neural plate and would form paired "hemineural plates" instead. Totipotent tissue from Hensen's node could pass into the gap between the two notochordal processes and form a number of different caudal tissue types.

spinal cord almost as far as S2 is formed by primary neurulation, and secondary neurulation involves formation of the terminal filum and maybe the lower sacral spinal cord.

Secondary Neurulation

After the caudal neuropore has closed on postovulatory days 25 to 27, the entire nervous system is covered with skin and more caudal neural development occurs by secondary neurulation. By this time, primitive streak remnants have regressed to form a caudal cell mass (CCM) at the caudal embryonic pole. This CCM extends from the posterior neuropore to the cloacal membrane and composed of multipotent cells.

The mechanism described for secondary neurulation is specific to each species. Human secondary neurulation closely resembles that of the mouse. As per Müller and O'Rahilly, neural cord here is in continuity with the primary neural tube. It has a single lumen, which is continuous with the central canal of the primary neural tube.

Lemire and Bolli believes there is a resemblance to the neurulation process of chick embryos. They described multiple independent secondary tubes with separate lumina and no identifiable connections with one another or the primary neural tube.

Occlusion of the Spinal Neurocele

Between postovulatory days 23 to 32 while the neural tube is closing and also after it, there is temporary occlusion of the central lumen, because of the apposition of the lateral walls of the neural tube. This occlusion starts cranially beyond the first pair of somites and goes as far caudally as the ninth somite involving 60% of the neuraxis. The neural tube located cranially beyond occlusion expands rapidly because of its growth and expansion of the ventricular system. The closed cranial neuropore and the occluded caudal neural tube helps in isolating the cranial ventricular system. This isolated ventricular system has an intraluminal pressure, providing a driving force for brain growth. One of the mechanisms responsible for Chiari malformation is the failure to maintain this driving force in patients with open neural tube.

Embryogenesis of Human NTDs

Von Recklinghausen proposed the nonclosure of neural tube theory for explaining the neural tube defects.

Pang et al proposed a unified theory on the embryogenesis of SCM and suggested the error occurs almost at the same time as the primitive neurenteric canal is closing. The "accessory neurenteric canal" is formed through the midline embryonic disc. This establishes the communication between yolk sac and amnion and is the basis of error. This accessory neurenteric canal allows continued contact between ectoderm and endoderm and results in regional "splitting" of the notochord and the overlying neural plate. Elsewhere, it is rolled up to form the neural tube. The site of the fistula is variable but always cranial to the primitive neurenteric canal and explains why all SCMs involve cord segments that are rostral to the coccyx. The normal neurenteric canal opens into the primitive pit, which lies opposite the coccyx. Endomesenchymal tract is formed by the condensation of mesenchyme around this abnormal fistula. This occupies the space between the split notochord and split neural plate and may contain precursor cells from the meninx primitiva. In the presence of meninx primitiva cells, there is the formation of a bony septum, resulting in type I SCM. In the absence of it, a fibrous band would result in the formation of type II SCM. The composite type of SCMs may form when there are multiple accessory canals (**Fig. 2.2**).

Generally, in SCM type I, a single bony spur arises from the posterior surface of the vertebral body. However, there may be occasions when the spur arises from the posterior arch. Chandra et al proposed two hypotheses for posterior origin of bony spur including: (1) ventral cell mass gets disconnected after dorsal migration of meninx primitiva cells, and (2) the other possibility being initial migration of meninx primitiva cells around the hemicords instead of between them to accumulate along the dorsal arch.

Others like Dias et al have recently proposed that problems in the midline axial integration during gastrulation may also be responsible for several myelomeningoceles. The Hensen node fails to lay down a single notochord. The authors suggest that during gastrulation each half of the Hensen node gives rise to paired notochordal analgen. On either side of the node, two independent hemineural plates grow

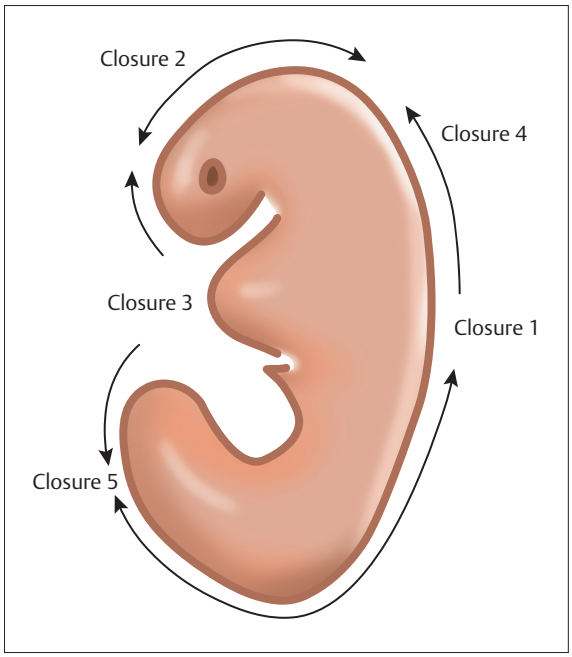

Fig. 2.2 Modified human concept based on mouse multisite closure, as used for retrospective interpretation of human neural tube defects (NTDs).

and subsequently develop into a hemicord. If neurulation fails in one hemicord, hemimyelomenigocele happens. If it fails in both the hemicords, it results in myelomeningocele associated with an SCM (**Fig. 2.3**). Multipotent cells contained within the Hensen node is responsible for the varied associated malformations like neurenteric cysts, combined spina bifida (split notochord syndrome), and other entities between the two hemicords.

Enough evidence supports genetic cause for neural tube defects (NTDs). The fact that the first-degree relatives of patients are at a high risk of NTDs is a pointer toward genetic involvement. The risk of the sibling having NTD is 2 to 3%, and with the third child, the risk increases to 10%. The incidence of NTDs is varied for different populations. Concordance rates vary between 3.7 and 18% in monozygotic twin pairs. NTDs may also be seen in association with known genetic and chromosomal anomalies (such as Waardenberg syndrome, Trisomy 13 and 18).

Variety of molecular involvements have been shown in NTDs including transcription factors and coactivators, signal transducers, folate binding proteins, tumour suppressor gene products, cytoskeletal components, DNA methyltransferases, nuclear and cell membrane receptors, chromosomal proteins, gap junction proteins, cell surface receptors, and actin regulators and binding proteins. These may be related to specific types of NTDs. Each may involve disruption of specific waves of neural tube closure and can be disrupted without interfering with other waves. Genes controlling folate metabolism and methyltransferase reactions involving methionine and homocysteine have caught recent attention. Supplementing periconceptional folate to pregnant women have shown reduction in the incidence of NTDs both in women known to have child with NTDs and without.

Almost half of NTDs may be caused by factors that are independent of folate mechanisms. Teratogens too may be contributing to various cellular mechanisms causing NTDs in humans. Valproic acid is known

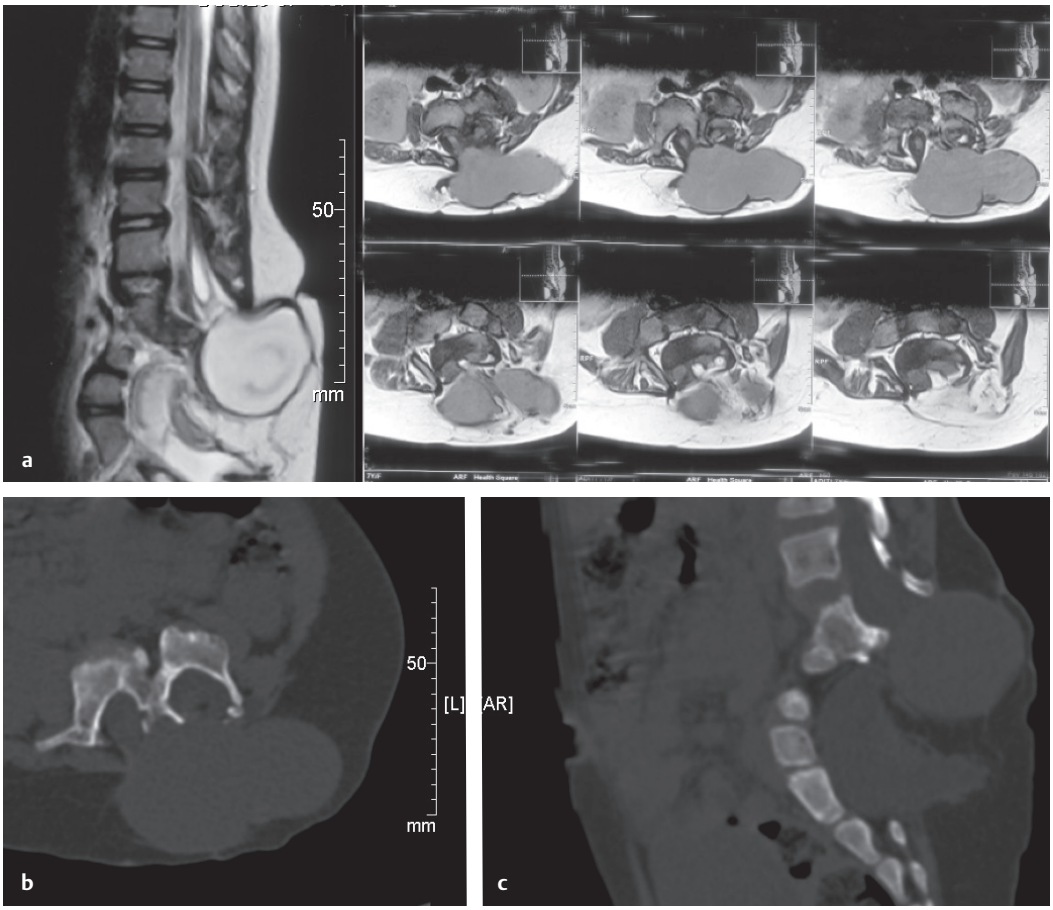

Fig. 2.3 (a–c) Failure in primary neurulation in both the hemicords results in myelomenigocoele associated with SCM type I.

to cause NTDs in humans. The most likely mechanism of teratogenic effect being inhibition of neural fold fusion, as the folate metabolic pathways is disrupted because of interference in conversion of tetrahydrofolate to 5-formyltetrahydro-folate.

Suggested Readings

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