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Abstract

Morphological Changes During Junctional Neurulation in Chick Embryo

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Background: Neural tube defect (NTD), which results from disturbances in neurulation, is one of the most common congenital anomalies in newborns. Ambivalent clinical cases of NTD suggesting a new phenotype have been reported. While conventional cases show errors in proper formation of neural tube, in this report both the primary and secondary neural tubes were fully developed but unconnected both distance and function wise. Hence, there have been speculations on a novel transition between primary and secondary neurulation termed junctional neurulation. This study investigates the morphological transition that connects the primary and secondary neural tubes during junctional neurulation to clarify the definition of

key terms and to characterize the morphology of the junctional zone in the chick embryo.

Methods: Chick embryos of Hamilton-Hamburger (HH) stages 14-28 were used. Collected embryos were embedded and sectioned. Morphology of the transition from primary neurulation to secondary neurulation was investigated in HH stages by Hematoxylin and Eosin (H&E) staining.

Results: Beginning at HH14, the lumen of the primary neural tube decreases and changes from an elongated shape to a round shape. At HH16, multiple cavitation activity is found at the caudal cell mass (CCM) and at HH18 are fused at the upper part of the secondary neural tube. In later stages from HH20 to HH28, widening of the lumen to the caliber of the primary neural tube and the width of the central canal is observed throughout the entire spinal cord.

Conclusions: Junctional neurulation occurs after the end of primary neurulation and overlaps with the cavitation and coalescence phase of secondary neurulation. It conjoins the primary and secondary neural tubes by conjoining the lumens to establish topological continuity. Its activity is evident at HH14, and continues until HH28. The junctional zone includes the area where primary and secondary neural tubes coexist, but there are limitations in defining the exact boundaries.

Key words: junctional neurulation, neural tube defect, chick embryo, secondary neurulation, primary neurulation, caudal cell mass

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List of abbreviations

CCM: Caudal Cell Mass

H&E: Hematoxylin & Eosin

HH: Hamburger-Hamilton

NSB: Node-Streak Border

NTD: Neural Tube Defect

Contents

Introduction

Two consecutive fundamental processes initiate development of the human neuraxis: primary neurulation and secondary neurulation. Primary neurulation gives rise to the neural tube, which is the primordium of the central nervous system. Neuroectodermal tissues consequent to gastrulation receive signals from the notochord to thicken and eventually ingresses to undergo the process of “rolling and folding” to shape the neural fold. Subsequently, complete closure of the neural fold results in a tube like shape and forms the neural tube [11]. Contrary to the preceding event, secondary neurulation commences from a dispersed layer of pluripotent mesenchymal cells that aggregate focally to form the condensed medullary cord. The cord then undergoes cavitation which ultimately enlarges the inner diameter of the newly formed tube. Secondary neural tube then undergoes regression to form the filum terminale [12].

Neural tube defect (NTD) is one of the most common congenital anomaly in newborns, and it arises from developmental errors during neurulation. It is known to affect 1 in every 1000 pregnancies. Severe types of the disease have detrimental effects in that newborns have to live with the nerve damage and loss of function. NTDs can be classified into its two distinct phenotypes, open and closed. Primary neurulation has been known to be involved with the open phenotype. Open spina bifida is a type of NTD where there is an opening in the spine. Depending on the level of spinal cord affected and its extremity, there may be lack of sensation, inability to walk, and incontinence. Other symptoms may include hydrocephalus, vertebral deformities, genitourinary and gastrointestinal disorders [2].

Secondary neurulation is responsible for the posterior end and therefore forms the lower spine at lumbar and sacral levels. It differs from primary neurulation in that it goes through 3 phases: condensation, cavitation, and regression [3]. It begins at the tail bud where a group of pluripotent cells begin to aggregate. This condensed bulk of cells is termed the caudal cell mass (CCM), which is known to have both mesenchymal and neural character [10]. It ultimately contributes to the somites, mesoderm, and the neural tube [6]. Progressively, cavitation activity happens in the CCM. Multiple cavities form then coalesce to form a single lumen [9]. In humans and in chick embryos, the secondary neural tube further elongates until it reaches a peak and undergoes regression. Since it forms from cavitation of the mesenchyme and does not involve folding of the neuroectoderm, anomalies of the secondary neurulation have intact skin.

As observed in caudal agenesis, the medullary cord, which is the condensed mesenchyme, may fail to form resulting in the absence of a conus. In other cases, a proper solid medullary cord may form but with malformations in the bone or muscle and often accompanied by lipoma, as in lumbosacral lipomas. Additionally, a proper cord may form but its regression may not take place as with cases of retained medullary cord. There is a wide spectrum of symptoms as some cases may be asymptomatic while some may result in urinary or bowel problems, or trouble in walking.

Although there has been significant progress in diagnosis and surgical treatment there is not yet an absolute cure. NTD has an obscure etiology as it is multifactorial; there is no single responsible gene. Exact mechanisms at cellular and molecular levels underlying

the failure of closure in the developing neural tube, and the spinal defects underneath the skin are mostly unknown. Thus, it is necessary to understand detailed mechanism of neurulation which is important in the interpretation of the pathology of NTDs [2].

Case studies by Pang et al. (2017) have reported a distinct phenotype unlike those previously mentioned. Three similar clinical cases mentioned were not accompanied by characteristic errors in neural tube formation. Instead, portions of the spinal cord arising from primary neurulation was near complete and fully functional, and the conus resulting from a properly tapered medullary cord during secondary neurulation was also completely normal. Furthermore, no skin defects were observed. The uniqueness lied in that the regions formed by primary and secondary neural tubes respectively were not conjoined. Instead, they were separated both function-wise and distance-wise [5]. Therefore, these newly reported cases were suggested to be classified under a new phenotype termed junctional neural tube defect, since it suggests errors in the conjoining process. It became necessary to understand the exact spatiotemporal mechanisms behind the conjoining process between primary and secondary neurulation to grasp an understanding of the underlying pathology of junctional NTD.

Although it is generally thought that open phenotype results from errors during primary neurulation and that closed phenotype results from errors during secondary neurulation, this is not definitive. It is inaccurate to state that the errors are mutually exclusive. As these developmental anomalies arise from two consecutive process with a shared purpose to ultimately form the spinal cord, some cases may naturally display traits implying mechanisms of both types of neurulation [1]. This is clearly illustrated in a case of dorsal spinal

cord lipoma where a premature disjunction during primary neurulation that results in complete closure of the skin [5].

Dady et al. (2014) elaborated junctional neurulation as a developmental program susceptible to NTDs. They discovered that not only does junctional neurulation functionally and spatially connect primary and secondary neural tubes, but it also supplies neural progenitors. They observed a region that was morphologically continuous with the primary neural tube but showed neurulation activity before notochord formation. It is well known that notochord is the precursor or primary neurulation, but it is formed after the secondary neural tube in secondary neurulation. Hence, this region demonstrated junctional neurulation activity as it is continuous with the primary neural tube but undergoing secondary neurulation activity. They defined this novel region of junctional neurulation activity as 'node-streak border (NSB)'. They also discovered that the cells of NSB undergo epithelium-to-mesenchyme transition (EMT) to form the junctional neural tube [4]. This was the first study on junctional neurulation, and it successfully explored changes in neural activity after the end of primary neurulation following the closure of the caudal neuropore. However, many questions still remain as activity after the onset of secondary neurulation and as the secondary neural tube is made and how it conjoins with the primary neural tube, was not investigated.

This study aimed to elucidate the process of junctional neurulation focusing on the morphological changes. Specifically, the process of how and when the lumen of primary and secondary neurulation are conjoined along with the changes in the morphology of the neural tubes and notochord were assessed [8]. We used chick embryos from Hamburger-Hamilton (HH) stages 14 to 28 as the

model of study because the anatomical region of the spinal cord that rises from secondary neurulation most closely resemble that of humans, and also because chick embryos undergo the process of tail regression like humans do [7]. Morphological traits were observed using Hematoxylin and Eosin (H&E) staining to identify the location and extent of junctional zone throughout development.

Material and Methods

Chick embryo samples

Eggs were purchased from a commercial supplier (Pulmuone, South Korea), and incubated in a turning incubator at 38'C with 65% relative humidity. Following incubation, chick embryos of desired HH stages were harvested. The embryos were fixed in 4% paraformaldehyde solution for overnight. At least four samples suitable for morphological evaluation were obtained for each stage.

H&E staining

Paraffin-embedded tissue sections of 4μm were stained with hematoxylin (Harris' hematoxylin solution, Merck, USA) for 5 minutes then immersed briefly in a 1% hydrochloric acid in ethanol, then with eosin (Eosin Y, BBC biochemical, USA) for 15 seconds. Stained tissue sections were mounted with Leica's CV ultra-mounting medium (Leica, Germany) then examined under a microscope.

Results

Definition of key terms

We define junctional neurulation as the process in which the lumina of the primary neural tube and the secondary neural tube are conjoined and topological continuity is established. Junctional neurulation should end as the continuity is completed. This will correspond to the time before the point when the secondary neural tube starts to generate. It is a separate process from primary and secondary neurulation and according to the abovementioned definition, it may start at the end of primary neurulation and overlap with some of the cavitation and coalescence phase of secondary neurulation.

The junctional neural tube may be defined as either a neural structure between the primary and secondary neural tubes which eventually disappears as secondary neurulation becomes fulminant, or a tube like structure formed by the connection of the lumen of primary and secondary neural tubes.

The junctional zone can be defined as the zone where junctional neurulation occurs, includes the area where the primary and secondary neural tubes coexist, and also the junctional neural tube. Previously ‘overlap zone’ or ‘junctional zone’ referred to the area where the primary neural tube and the secondary neural tube coexisted, the former in the dorsal and the latter in the ventral area of the body. The concept of junctional neurulation was not considered when defining this term.

Morphology of primary neural tube, secondary neural tube and the notochord

We first examined the morphology of HH14 chick embryos when primary neurulation is complete, marked by fully closed neural grooves at the dorsal end of primary neural tube (Fig 2A). Observing the embryo in axial orientation and moving caudally towards the tail of the embryo, primary neural tube begins to get smaller in size and so does its lumen. At this point, the primary neural tube occupies the dorsal half of the embryo and the ventral portion is composed of CCM (Fig 2D, green arrow), hence forming the junctional zone. Near the tail, the ventral half of the embryo is composed of the CCM (Fig 2F, yellow star). The border between the primary and secondary neural cannot be clearly delineated in the H&E sections, but the difference in the pattern of cellular arrangement between the two neural tubes suggest their coexistence. In the primary neural tube the cells surround the lumen in a pseudostratified columnar fashion, whereas the CCM is a mass of randomly placed cells. The change in the morphology of the notochord also implies that the ventral portion is caudal cell mass. The notochord loses its round shape (as clearly seen at the level of the typical primary neural tube, Figure 2A), becomes closer to and eventually fuses with the ventral portion of the neural tube (Figure 2C, red arrow). The lumen of the primary neural tube decreases in diameter with change of the shape from the typical ovoid shape (Figure 2A) to a round appearance (Figure 2E).

In HH16 embryos, as the primary neural tube decreases in size in the junctional zone, the caudal cell mass increases in proportion and invades the primary neural tube into its lumen (Figure 3C, red arrow). The notochord is similar to how it was seen in HH 14.

Multiple cavities start to form in the caudal cell mass (Figure 3F, 3G; round circles)

In HH18, the portion of the primary neural tube in the junctional zone is decreased even more. As the tail gut (Figure 4C, bracket) is formed, the notochord becomes separated from the caudal cell mass in the junctional zone (Figure 4C, 4D, asterisks). The cavities fuse with end of primary neural tube lumen (Figure 4C green arrow, 4D red arrow).

In HH20, the junctional zone is dominated by the secondary neural tube, similar to the previous stage. The most noted morphology in this stage is the clear fusion (Figure 5C, red arrow) of the primary neural tube lumen and the cavities (Figure 5D, green arrow) of the secondary neural tube.

In HH24 the fusion (Figure 6E, green arrow) of the most tapered, caudal end of the primary neural tube lumen (Figure 6D, red arrow), and the cavities of the secondary neural tube (Figure 6F, yellow arrow) is seen.

It seems that at HH 28, the primary neural tube is completely connected with the secondary neural tube lumen, as there's no decrease or 'tapering' of the caliber of the lumen. The lumen eventually gets smaller and separates into cavities (Figure 7D green arrow), as the process of secondary neurulation is not finished. The primary neural tube gets smaller at the caudal end and separates into distinct cavities.

Schematics of junctional neurulation

Junctional neurulation is the process in which the topological continuity is established between the primary and secondary neural tube was assessed. As the caudal end of primary neural tube tapers, the secondary neural tube, located ventral to the primary increases in size. The lumen of primary neural tube also decreases in size, and changes the shape from elongated to round. Meanwhile, multiple cavities are formed in the caudal end of CCM and coalescence of the cavities is found in the upper part of the secondary neural tube. One of the upper cavities fuses with the small, tapered-end of the primary neural tube lumen. As the two lumens are fused, the lumen widens to the caliber of the primary neural tube and the width of the central canal is established throughout the entire spinal cord. This marks the end of junctional neurulation.

Figure 1.

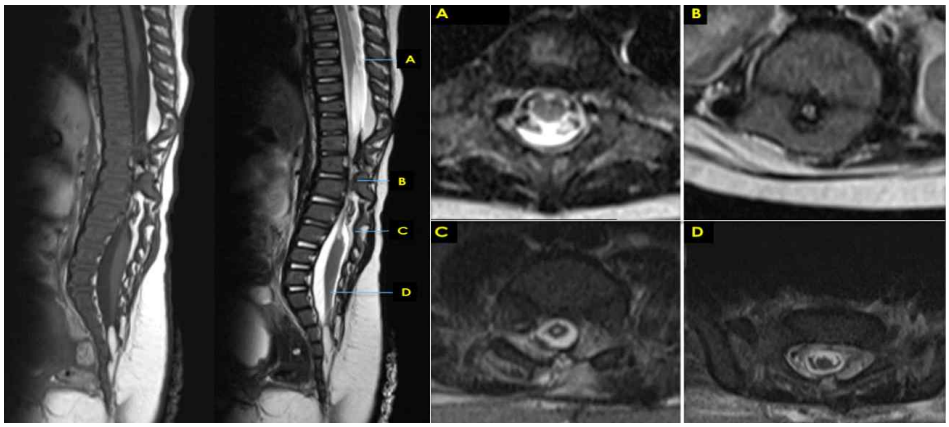

Figure 1. Pre-operative spinal MRI of a patient with junctional neural tube defect.

(A): Normal upper spinal cord at mid-thoracic level. **(B):** Spinal cord begins to narrow from T10. **(C):** Until L2 spinal cord remains a thin band. **(D):** From L2 to the conus the spinal cord appears normal again.

Figure 2.

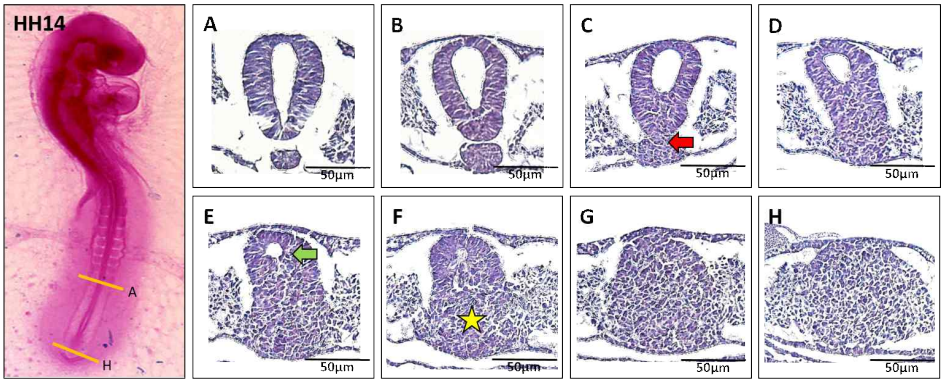

Figure 2. Morphology of HH14 chick embryo from the primary neural tube to the tail bud.

(A): complete primary neural tube of HH14. **(B)**: The primary neural tube shows movements in the ventral end where the neural cell population in the ventral end has thickened and the lumen has regressed in size. The notochord, compared to the rostral end 1A, has increased in size. The gap between the neural tube and the notochord appears to decrease. **(C)**: the lumen has decrease even more in size and the boundary between the notochord and the neural tube is no longer visible. **(D-E):** The lumen has further decreased in size and adopts a rounder shape. The notochord and the neural tube is no longer distinguishable. **(F):** The remnants of the lumen are barely visible. **(G-H)**: CCM of HH14. **(red arrow):** indicates region of secondary neurulation activity. **(green arrow)**: indicates tapered lumen of the primary neural tube. **(yellow star):** indicates CCM.

Figure 3.

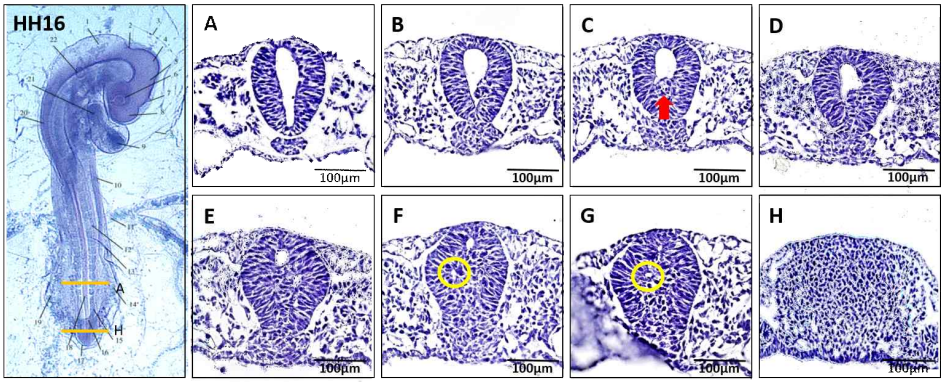

Figure 3. Morphology of HH16 chick embryo from the primary neural tube to the tail bud.

(A): complete primary neural tube of HH16. **(B)**: The notochord has thickened and the gap between the notochord and the neural tube has disappeared. The lumen has decreased in size and the ventral end of the neural tube starts protruding into the lumen. **(C-D):** The lumen's characteristic linear diamond-shape is replaced as the robust activity found in the ventral end of the neural tube makes the neural cells to protrude into the lumen. **(E-F):** The lumen is present but much smaller. Small multiple cavities are present. **(G-H)**: CCM of HH16. **(red arrow):** indicates region of secondary neurulation activity. **(yellow circles):** indicates cavitation.

Figure 4.

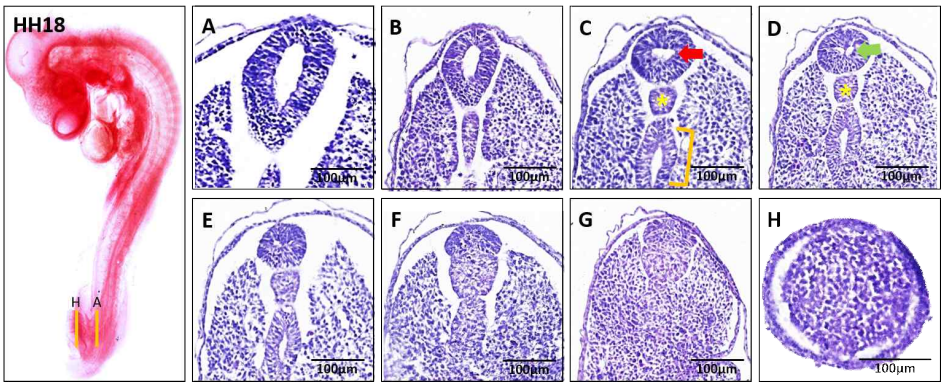

Figure 4. Morphology of HH18 chick embryo from the primary neural tube to the tail bud.

(A): complete primary neural tube of HH18. **(B-C)**: the neural tube becomes smaller and rounder in shape. **(D-E):** The lumen is separated into two distinct cavities that eventually decrease in size. **(F):** The cavities have disappeared. The notochord has enlarged in size and the gap between primary neural tube and notochord has disappeared. **(G):** The neural tube portion and the notochord portion are roughly identifiable but boundaries have disappeared. **(H)**: CCM of HH18. **(red arrow):** lumen of primary neural tube. **(green arrow)**: indicates separation of the lumen into cavities. **(yellow brackets):** tail gut. **(yellow asterisk):** notochord.

Figure 5.

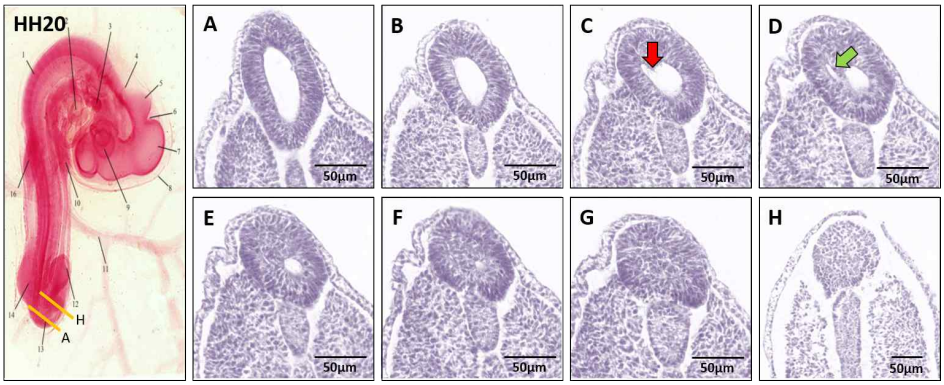

Figure 5. Morphology of HH20 chick embryo from the primary neural tube to the tail bud.

(A): complete primary neural tube of HH20. **(B)**: neural tube has decreased in size **(C)**: the dorsal end of the neural tube gets thicker and protrudes into the lumen. The middle part of the dorsal end protrudes slightly further in. **(D):** The middle portion appears to cross the lumen, separating it into two distinct cavities. **(E-F):** The left cavity disappears and the right cavity remains, and gradually decreases in size. The notochord now meets the neural tube. **(G):** The cavities have completely disappeared. **(H):** The notochord and the CCM. **(red arrow):** indicates the septum which protrudes into the lumen to divide it into cavities. **(green arrow)**: indicates separation of the lumen into cavities.

Figure 6.

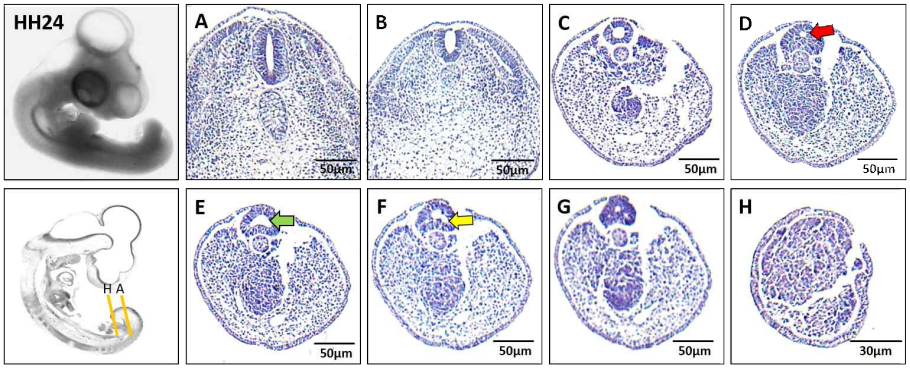

Figure 6. Morphology of HH24 chick embryo from the primary neural tube to the tail bud.

(A): complete primary neural tube of HH24. **(B)**: The narrow lumen of primary neural tube has slightly widened and become rounder in shape. **(C)**: The neural tube is round in shape and so is the notochord. The CCM is clearly distinguishable as a dark mass in the ventral end. **(D):** The CCM has increased in size. The neural cells of the neural tube have thickened and the lumen is now a small, narrow hole. The neural tube has adopted a bean-like shape. **(E):** The lumen has expanded into a bean-like shape. **(F-G)**: The lumen has again dissociated into three distinct cavities that decrease in size. **(H):** the CCM of HH24. **(red arrow):** indicates the tapered lumen of the primary neural tube that is now round in shape. **(green arrow)**: indicates the bean-shaped neural tube. **(yellow arrow):** indicates separation into cavities.

Figure 7.

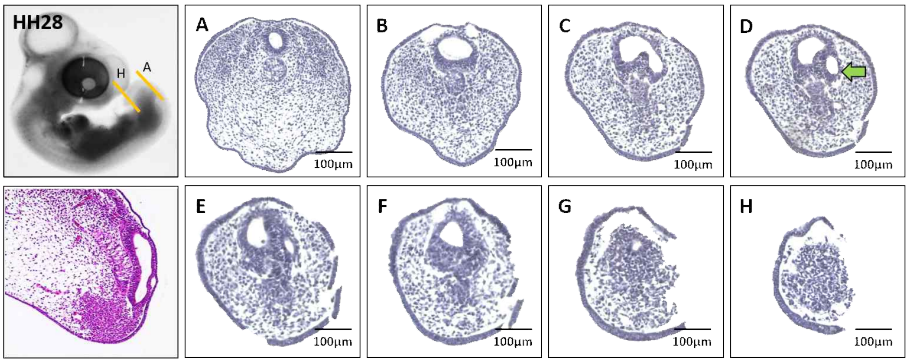

Figure 7. Morphology of HH28 chick embryo from the primary neural tube to the tail bud.

(A): complete primary neural tube of HH28. **(B):** The primary neural tube has become rounder in shape and the lumen has expanded horizontally. **(C):** The lumen of the neural tube adopts a bean-like shape due to the middle portion of the ventral end of the neural tube protruding into the lumen. **(D):** The middle portion has protruded completely into the dorsal end of the neural tube separating it into two distinct cavities. **(E):** The midline that separates the two cavities has thickened and is now continuous with the CCM. **(F-G):** The right cavity has disappeared. The left cavity gradually diminishes. **(H):** the CCM of HH28. The tapered lumen of the primary neural tube that is now round in shape. **(green arrow):** indicates the bean-shaped neural tube.

Figure 8.

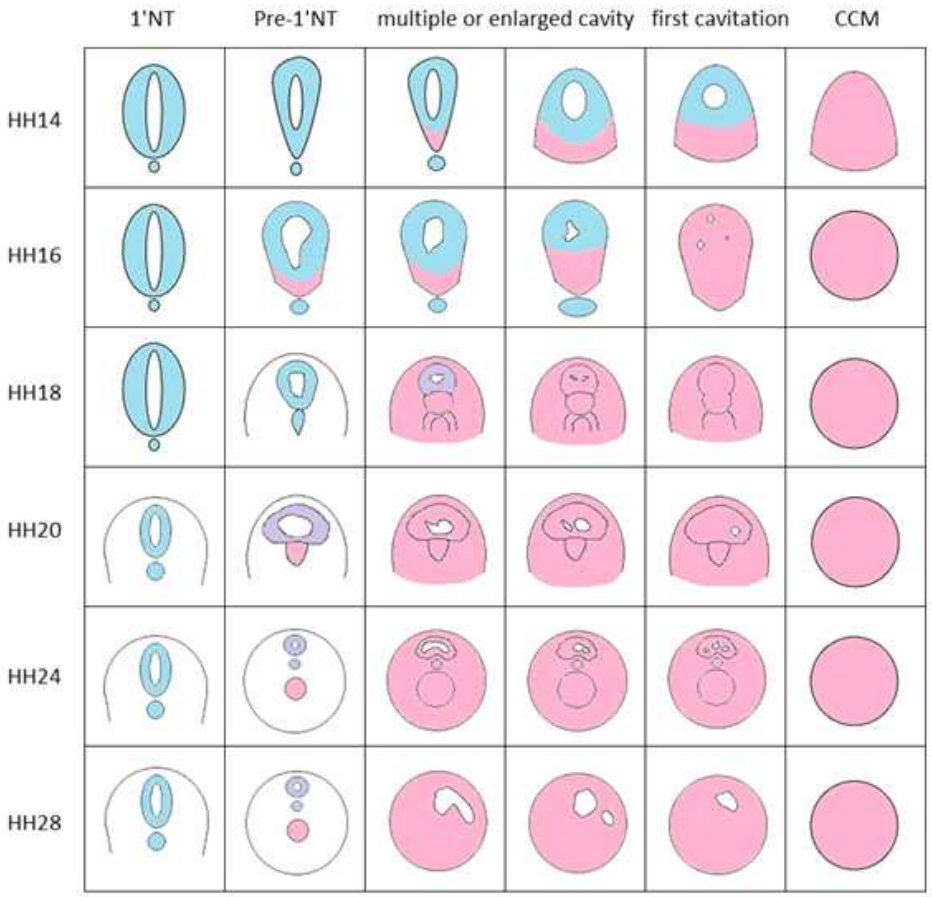
1'NT
Pre-1'NT
multiple or enlarged cavity
first cavitation
CCM
HH14
HH16
HH18
HH20
HH24
HH28

Figure 8. Schematics of the morphological change during junctional neurulation.

Pink: indicates portion of the embryo formed from secondary neurulation. **Blue** indicates regions formed by primary neurulation. **Purple:** indicates regions formed by either primary or secondary neurulation, or both. Rostral portion of the embryo from the primary neural tube until when the primary neural tube begins to tether and appear narrower show contributions mostly from primary neurulation. Regions of cavitation and the CCM show contributions mostly from secondary neurulation.

Discussion

It is critical to define the junctional zone to be able to predict exact levels of the spinal cord susceptible to junctional neural tube defects. To understand the developmental errors during junctional neurulation that result in congenital anomalies of the spine, it is necessary to understand the mechanisms taking place. To the best of our knowledge, this is the first to demonstrate the morphological changes taking place after the onset of secondary neurulation in the junctional zone.

We used chick embryos from HH stages 14 to 28 because at HH14 the caudal neuropore closes and primary neurulation ends [4]. Therefore, we hypothesized that junctional neurulation activity would begin here. We observed until HH28 when chick embryo's tail bud mesenchyme, known as the CCM, starts to diminish. Since CCM is known to be a key contributor to secondary neurulation, we hypothesized that at this stage secondary neurulation is near its end and tail regression activity begins [12]. Morphological traits were observed using H&E staining to identify the location and extent of junctional zone throughout development. We focused on the morphological changes in the CCM. We also concentrated on the development of the notochord as in secondary neurulation notochord forms after the formation of the secondary neural tube unlike in primary neurulation where notochord is a precursor [8]. We observed the morphology of the neural tube lumen throughout development as we hypothesized that the two neural tubes would share a lumen once completely connected. Through investigating these morphological changes throughout developmental stages of chick embryo, we aim to

define junctional neurulation.

The morphology of junctional neurulation is unique in that the two separately formed neural tubes are later conjoined by the lumen. The lumen of the primary neural tube tapers near the caudal end of the chick embryo with the beginning of secondary neurulation activity. As the secondary neural tube is formed from the cavitation of CCM, the newly formed secondary neural tube first appears to have a round lumen shape at the very caudal end. The narrow round lumen of the secondary neural tube then meets with the tapered lumen of the primary neural tube. The complete fusion of the two lumen marks the end of junctional neurulation.

Our study on junctional neurulation is significant in that other than Dady's work in 2014 there have been no recent studies elucidating junctional neurulation. There have been observations in the past of an "overlap zone" by Criley in 1969 on the analysis of the embryonic sources and mechanisms of development of posterior levels of chick neural tubes. This paper elucidated the overlap zone as an area of multiple cavitation and a single consistent dorsal cavity. It observed that multiple cavitation coalesced to form a single lumen [3]. Another study on the ultrastructure of secondary neurulation by Schoenwolf in 1980 that mentioned the term "overlap zone". This overlap zone was suggested to connect the two neurulation processes. Multiple cavitation activity was observed in HH15 embryos and the authors suggested that the very dorsal cavity was part of the primary neural tube, much consistent with our study [10].

Previous studies on junctional neurulation by Dady et al (2014) have mentioned that junctional neurulation is a unique program that shapes a discrete region of the spinal cord susceptible to this new phenotype of neural tube defect. The presence of a node-streak border (NSB) that contributes to the junctional portion of the spinal cord up until the end of primary neurulation when caudal neuropore closes has been reported. But, the morphological changes in the chick embryo that parallel the contribution during the transition to secondary neurulation has not been yet clearly elucidated. Prickle-1, a gene involved in establishing planar cell polarity, has been suggested to be a key player in the switch from primary to secondary neurulation. Among many known molecular players involved in primary neurulation, Prickle-1 was notable in that its mutation would result only in local spinal dysraphisms. It was discovered that consistent with the hypothesis, Prickle-1 was found in the NSB but not in the primary neural tube. When Prickle-1 activity was impaired in the NSB, it resulted in chick embryos with malformed caudal neural tubes with no visible lumens [4]. However, this was only visible at HH9 and hence to validate these results it is necessary to examine the activity of Prickle-1 at later stages such as HH14 when robust secondary neurulation activity is clearly visible. Much remains yet to be investigated as there was mention of the overlap zone on past papers and a sudden discovery of junctional neurulation in 2014 along with clinical reports on junctional NTDs. The gap between the observation of overlap zone and the recent study on junctional neurulation suggests many blanks that need to be filled and numerous future implications.

CCM is known to be a key player in secondary neurulation, but the vastness of its contribution is not precisely known. CCM undergoes condensation, cavitation, and regression during the process of secondary neurulation. It is known to have both mesenchymal and neural character [1]. However, there is controversy on whether it is composed of mesenchymal and neural stem cells, or whether it is a neuromesodermal progenitor that adopts either mesodermal or neural fate. This is not exactly known up to this date. It ultimately contributes to the neural tube and the somites, but there have been several inconclusive hypotheses on its contributions to the endoderm and therefore other organs [9]. Also, the mechanisms of secondary neurulation that result in the closed NTD is not clearly identified morphologically, or genetically. Unlike primary NTDs, no key molecular contributors have been defined. Much of secondary neurulation is unstudied and remains a mystery, like molecular players, ultimate contributions, the exact beginning and the end, and how it is regressed. Future studies are necessary for an intricate understanding of the process and its underlying mechanisms to define the causative genes and activities of closed NTDs.

Further studies are necessary to accurately discriminate primary neural tube related regions and secondary neural tube related regions. There were limitations in this paper as the exact boundaries of the junctional zone remains unclear. Also, the later aspect of secondary neurulation, the regression of the tube, was not fully investigated. Suggested future studies on this topic include immunostaining, and 3D CT imaging of the junctional zone.

Taken together, our results provide a premature morphological insight on junctional neurulation. Here it is susceptible to a junctional phenotype of neural tube defect and further studies in the mechanism of the change in character of the cell population will provide a better understanding into understanding mechanisms of this congenital anomaly.

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국 문 초 록

목적: 신경배형성시 발생하는 어긋남으로 인해 일어난다고 알려진 신경관 결손 (NTD)은, 신생아에서 매우 흔히 발견되는 선천성 기형이다. 기존 신경관결손에 대한 임상 보고는 신경관 형성에 문제가 발생한 경우가 대부분이었으나, 본 연구는 1차신경관과 2차신경관이 올바르게 형성 되었지만 그의 연결이 형태학적으로 또는 기능적으로 안된 임상 사례들에 대한 기초연구이다. 따라서 본 연구의 신경관 결손 사례는 1차신경관과 2차신경관을 연결하는 과정인 '접합부신경배형성 (junctional neurulation)' 이라는 과정에서 이형성이 일어났을 것이라고 사료되었다. 본 연구에서는 닭 배자의 신경관 접합 과정에서 1차신경관과 2차신경관의 결합 부위의 형태학적 변이를 관찰함으로써 닭 배자의 결합부위에서 발생하는 접합부신경배형성을 시간적으로 정의하고자 한다.

방법: Hamburger-Hamilton (HH) stage 14-28의 닭 배자들을 파라핀에 심어 절편을 만들고, Hematoxylin & Eosin 염색을 통해 일차 신경배형성과 이차 신경배형성을 형태학적으로 관찰하였다.

결과: 1차신경관의 가늘고 긴 형태의 내강이 HH14에서부터는 둥그런 형태의 내강으로 바뀌면서 크기가 줄어든 것을 관찰하였다. HH16에서 관찰된 미부세포괴의 다발성 내강이HH18에서는 2차신경배에 윗부분에서 결합하는 현상을 보였다. HH20부터HH28의 척수전체에서1차신경관의 직경과 중심관의 너비에 맞춰 내강이 확장되는 것을 관찰되었다.

결론: 신경관의 위상적인 연속성을 형성하기 위해 1차신경관과 2차신경관의 내강을 결합하는 과정인 접합부신경배형성은 1차신경배형성의 완료 시점에 HH14에 시작해서 2차신경배형성의 공동이 형성되어 융합되는 단계인 HH28까지 동시에 발생한다. 접합 부위는 1차신경관과 2차신경관이 공존하는 부위 지만 정확한 경계를 정의하는 것에 대한 한계점이 있다.

주요어: 접합부신경배형성, 신경관 결손, 닭 배자, 2차신경관형성, 1차신경관형성, 미부세포괴

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