

Tomoko Nakatsu · Chigako Uwabe · Kohei Shiota

Neural tube closure in humans initiates at multiple sites: evidence from human embryos and implications for the pathogenesis of neural tube defects

Accepted: 14 December 1999

Abstract The closure of the neural tube (NT) in the human embryo has generally been described as a continuous process that begins at the level of the future cervical region and proceeds both rostrally and caudally. On the other hand, multiple initiation sites of NT closure have been demonstrated in mice and other animals. In humans, based on the study of neural tube defects (NTD) in clinical cases, van Allen et al. (1993) proposed a multi-site NT closure model in which five closure sites exist in the NT of human embryos. In the present study, we examined human embryos in which the NT was closing (Congenital Anomaly Research Center, Kyoto University) grossly and histologically, and found that NT closure in human embryos initiates at multiple sites but that the mode of NT closure in humans is different from that in many other animal species. In addition to the future cervical region that is widely accepted as an initiation site of NT closure (Site A), the mesencephalic-rhombencephalic boundary was found to be another initiation site (Site B). The second closure initiating at Site B proceeds bidirectionally and its caudal extension meets the first closure from Site A over the rhombencephalon, and the rostral extension of the second closure meets another closure extending from the rostral end of the neural groove (Site C) over the prosencephalon, where the anterior neuropore closes. The caudal extension of the first closure initiating at Site A was found to proceed all the way down to the caudal end of the neural groove where the posterior neuropore is formed, indicating that in humans, NT closure does not initiate at the caudal end of the neural groove to proceed rostrally. Since there is a considerable species difference in the mode of NT closure, we should be careful when extrapolating the data from other animals to the human. It seems that the type

of NTD affects the intrauterine survival of abnormal embryos. Almost all the embryos with total dysraphism appear to die by 5 weeks of gestation, those with an opening over the rhombencephalon by 6.5 weeks, and those with a defect at the frontal and parietal regions survive beyond 7 weeks.

Key words Neural tube closure · Multi-site closure model · Neural tube defects · Human embryo

Introduction

The neural tube (NT), the embryonic forerunner of the brain and spinal cord, is formed by the fusion of bilateral neural folds in the midline during the 4th week of gestation in human embryos. Neurulation is a complex process that involves various morphogenetic events such as proliferation of neural precursor cells (neuroblasts or matrix cells), differential development of the neuroepithelium and surface ectoderm, formation of the median hinge point in the neural plate, apical constriction of neuroepithelial cells, and expansion of the mesoderm and extracellular matrices (Schoenwolf and Smith 1990; Gilbert 1997).

In standard textbooks of human embryology, the closure of the NT in human embryos is described as the process that begins in the region of the future neck (between the third and sixth somites) and proceeds bidirectionally toward the cranial and caudal ends like a zip fastener (“continuous closure model”). The closure is completed when the anterior and posterior openings of the NT (anterior and posterior neuropores) are closed around 24 and 28 days, respectively, after fertilization (O’Rahilly and Müller 1994). The failure of the anterior neuropore to close results in cranial neural tube defects (NTD) such as anencephaly and encephalocele and that of the posterior neuropore in spina bifida and myelo[meningo]cele.

Recently, however, this continuous closure model of the human NT has been challenged. Based on the study of human clinical cases of NTD, van Allen et al. (1993)

T. Nakatsu · C. Uwabe · K. Shiota (✉)
Congenital Anomaly Research Center,
Department of Anatomy and Developmental Biology,
Graduate School of Medicine, Kyoto University,
Kyoto 606-8501, Japan
e-mail: kshiota@med.kyoto-u.ac.jp
Tel.: +81-75-753-4341, Fax: +81-75-751-7529

extrapolated the data of NT closure in mouse embryos to the human and proposed a "multi-site closure model" where five closures (four in the head and one in the lumbar region) occur during NT closure in the human embryo. According to their model, "closure 1," the first fusion between the apposed neural folds, occurs in the prospective cervical region and progresses both rostrally and caudally; "closure 2" takes place at the junction between the prosencephalon and mesencephalon and also extends bidirectionally; "closure 3" initiates at the rostral tip of the neural groove and proceeds caudally to meet "closure 2"; "closure 4", between "closures 1 and 2", covers the rhombencephalon and completes the closure of the cranial neural tube. "Closure 5" initiates from the caudal end of the neural groove and spreads cranially to meet "closure 1" thereby closing the posterior neuropore. Van Allen et al. (1993) claimed that this multi-site closure model is a working explanation for the pathogenesis of various types of human NTD as well as the frequency of associated structural anomalies. This intermittent or multi-site closure model was derived from the sequential observation of rodent embryos (Golden and Chernoff 1983; MacDonald et al. 1989; Sakai 1989; Juriloff et al. 1991, Golden and Chernoff 1993), but it is not clear whether the NT closes in a similar manner in human and rodent embryos.

Although NT closure is usually described as a self-evident phenomenon, the closure of the anterior and posterior neuropores in human embryos has actually not been examined in detail to date, except in a study by Müller and O'Rahilly (1986). This is probably because the closure of neuropores is a very rapid event and because human embryo specimens at the neurulation stage have only rarely been available.

The Kyoto Collection of Human Embryos (Nishimura 1975; Shiota 1991) has provided us with a unique opportunity to directly observe a substantial number of human embryos at the stage of neurulation. In the present study, we examined 68 externally normal human embryos at Carnegie stages 10–12 (CS10–12) by gross and histological methods in order to observe the normal neurulation process in the human and to test the hypothesis that the multi-site closure of the NT occurs in the human. Another 98 embryos with cranial and/or caudal NTD (CS 11–23) were also examined, and the pathogenesis of NTD and the genesis of their phenotypic heterogeneity are discussed.

Materials and methods

The human embryos studied were from the Kyoto Collection of Human Embryos held at the Congenital Anomaly Research Center of Kyoto University. The embryo collection consists of approximately 44,000 embryos that have been collected from an extensive area of Japan. Most of the embryos were obtained following termination of pregnancy given to healthy women for social reasons. A smaller number of cases were obtained from spontaneous abortions or after termination following maternal signs of threatened abortion. The age of most embryos in the collection was within 8 weeks after fertilization. Since the obstetricians did not examine

the aborted embryos in detail and sent them to our laboratory without any prior selection, the collection of specimens was not biased by the phenotype of the embryos. Further details of the embryo collection and its demographic characteristics have been previously described elsewhere (Nishimura 1975; Matsunaga and Shiota 1977; Shiota 1991).

The aborted embryos were fixed in 10% formalin or Bouin fluid soon after procurement, and after being sent to the laboratory in Kyoto University, they were staged (O'Rahilly and Müller 1987), measured, and examined for structural abnormalities and signs of intrauterine death under a dissection microscope. Some of the well-preserved embryos were photographed and made into serial histological sections.

In the present study, to observe the mode of NT closure in humans, externally normal human embryos with a fusing NT (Carnegie stages 10–12, $n=68$) and embryos with NTD (Carnegie stages 11–23, $n=98$) were examined. NTD embryos associated with holoprosencephaly were not included in the present study.

Results and discussion

The normal neurulation process in human embryos

Normally, NT closure in human embryos commences at CS10 (ca. 22 days after fertilization) and is completed by CS12 (ca. 26 days; Müller and O'Rahilly 1985, 1987). A representative selection of human embryos with the closing NT is presented in Fig. 1.

Under a dissecting microscope, the process of NT closure was examined in 15 embryos at early CS10, 8 at late CS10, 15 at CS11, and 30 at CS12. In early CS10 embryos with 4–6 somite pairs, the neural groove was deep and was open throughout its entire length (Fig. 1a). The rostral part of the neural plate indicative of the brain primordium was relatively flat and appeared wider and thicker than the more caudal part. Out of eight embryos at late CS10 in the present series (7–12 somite pairs), five cases were at an early stage of NT closure. The fusion of apposed neural folds began at late CS10 at the level of the future neck (Site A) and the NT was widely open at the more cranial and caudal parts. By the time NT closure began, the primordial prosencephalon, mesencephalon, and rhombencephalon were already demarcated (Fig. 1b).

The remaining three embryos at late CS10 had 10 somite pairs, and their neural folds had fused for the length of about 10 somite segments (Fig. 1c). By careful examination of these three embryos, the second initiation site of NT closure was recognized at the boundary between the mesencephalon and rhombencephalon (Site B), in addition to the first closure initiating at Site A at the prospective cervical level (Fig. 2). Thus, it was confirmed that NT closure in human embryos does initiate at multiple sites.

Cranial to the second closure initiating at Site B, the medial walls of the neural groove were found to appose to each other around the transition between the prosencephalon and mesencephalon, which is indicated by an arrowhead in Fig. 2. Although the medial walls appose and make contact with each other, the fusion of neural folds was never found to initiate at the mesencephalic-

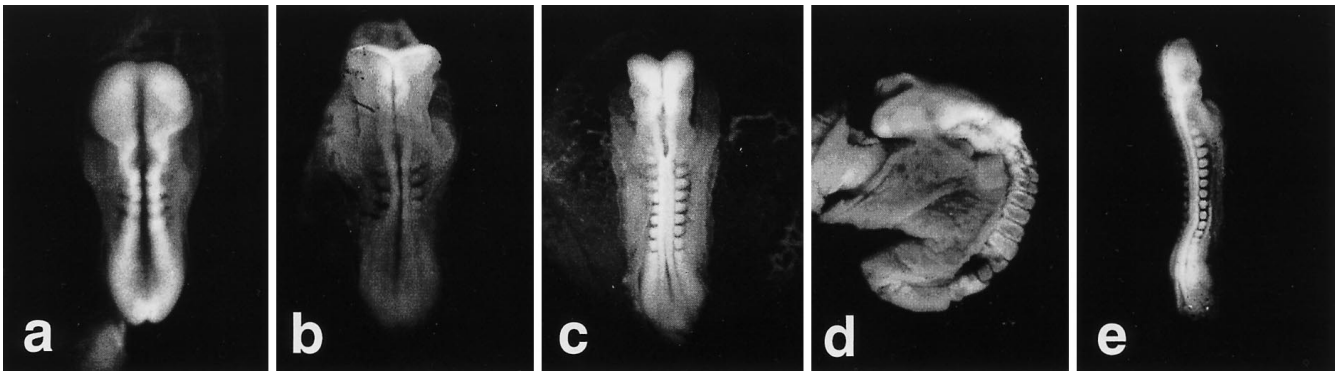


Fig. 1 a–1 Neurulation in human embryos. **a** A 5-somite embryo at early Carnegie stage (CS) 10 (no. 50823). The neural groove is deep and is open throughout its entire length (length of the embryo 2.0 mm). **b** A 5-somite embryo at late CS 10 (no. 23578). The primordial prosencephalon, mesencephalon, and rhombencephalon are already demarcated, and the neural folds begin to appose at several sites (length 2.2 mm). **c** A 10-somite embryo at late CS 10 (no. 33222). The NT closure has begun and the neural folds are

fused for the length of eight somite segments. The NT is widely open more rostrally and caudally (length 2.8 mm). **d** A 13-somite embryo at CS 11 (no. 13823). The NT is open over the mesencephalon and prosencephalon and at lumbosacral levels (greatest length 1.9 mm) **e** A 14-somite embryo at CS 11 (no. 4354). The NT closure is nearly completed, but the anterior and posterior neuropores are still clearly recognized over the prosencephalon and at the lower end of the NT, respectively (length 3.6 mm)



Fig. 2 A 10-somite embryo at CS 10 with the closing NT (no. 20297). The bilateral neural folds are fusing at the future cervical region. Another initiation site of NT closure is observed at the level of mesencephalic-rhombencephalic transition (*arrow*). The medial walls of the mesencephalon appear to make contact with each other (*arrowhead*), but they do not adhere to each other and NT closure does not initiate here

rhombencephalic boundary. In the chick embryo, a similar apposition of the medial walls of the mesencephalic neural groove has been observed to precede the fusion of neural folds (van Straaten et al. 1997). They postulated that such an apposition of the medial walls of the neural groove may avoid the possibility of mismatch of the neural folds. The medial walls of the neural groove do not adhere to each other, probably because appropriate adhe-

sion molecules necessary for epithelial fusion are expressed only at the neural folds.

The opening between the first and second closures is as small as the length of 3–4 somite segments and therefore this small slit-like opening may exist only briefly, probably for a “window” of a few hours. In embryos at a slightly more advanced stage of neurulation, the NT remained open continuously over the mesencephalon and prosencephalon (Fig. 1d). In none of the embryos at this stage, was an initiation site of NT closure observed at the boundary between the prosencephalon and mesencephalon, nor were there separate openings of the NT over the prosencephalon and mesencephalon as observed in rodent embryos (Juriloff et al. 1991).

Figure 3 shows serial transverse sections of the anterior part of the prosencephalon in a CS11 embryo with 13 somite pairs (embryo No. 4354), in which the NT was still open over the prosencephalon and at the caudal end (its gross appearance is shown in Fig. 1e). Near the rostral end of the anterior neuropore, the terminal lip of the neuropore was formed by the fusion of the most rostral part of the bilateral neural folds (Fig. 3d) and more caudally the neural folds were approaching the midline (Fig. 3e). This histological finding suggests that NT closure in the human initiates also at the rostral tip of the neural groove (Site C) and moves caudally to some extent.

Figure 4 shows the anterior neuropore formed near the rostral end of the prosencephalon where the caudal extension of the closure initiating at Site C meets the one starting at Site B and extending rostrally over the mesencephalon and prosencephalon. The closure of the anterior neuropore is therefore a bidirectional event. Müller and O’Rahilly (1986) reported a similar finding that at CS11, one fusion of the neural folds initiates at the prospective cervical region and proceeds rostrally over the midbrain and diencephalon, and another fusion starts simultaneously from the telencephalic region and extends cau-

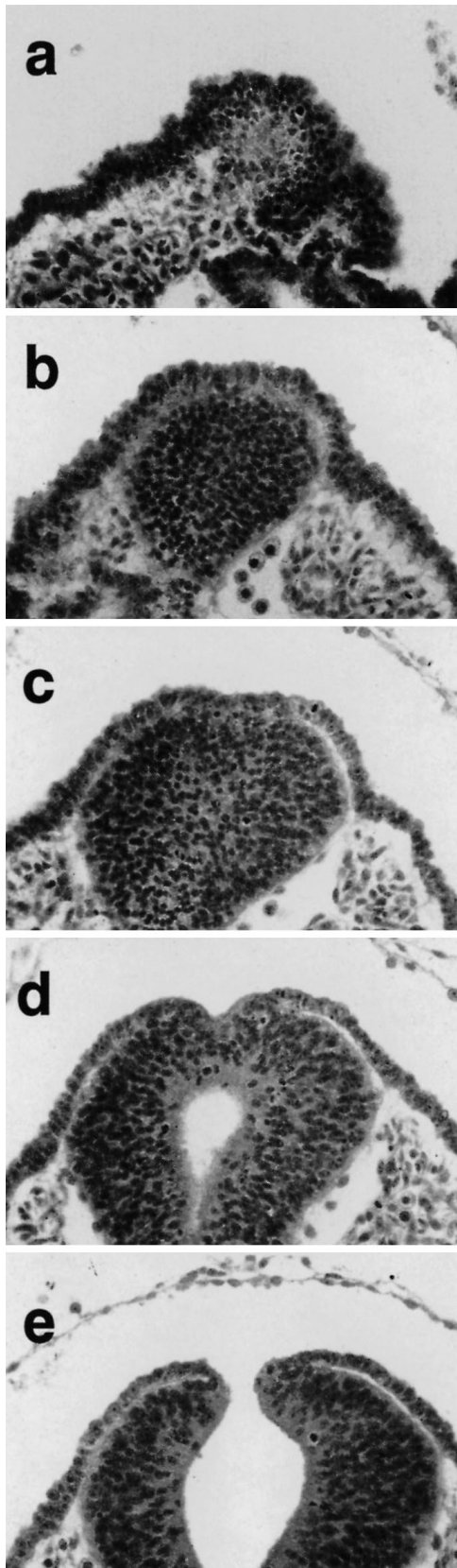


Fig. 3 Serial transverse sections of a CS11 embryo (no. 4354) through the anterior part of the prosencephalon in a rostro-caudal sequence (a–e). In **d**, the lumen of the prosencephalon is recognized, and the fused neural folds form the terminal lip of the anterior neuropore. More caudally (e), the neural folds are approaching to make contact with each other

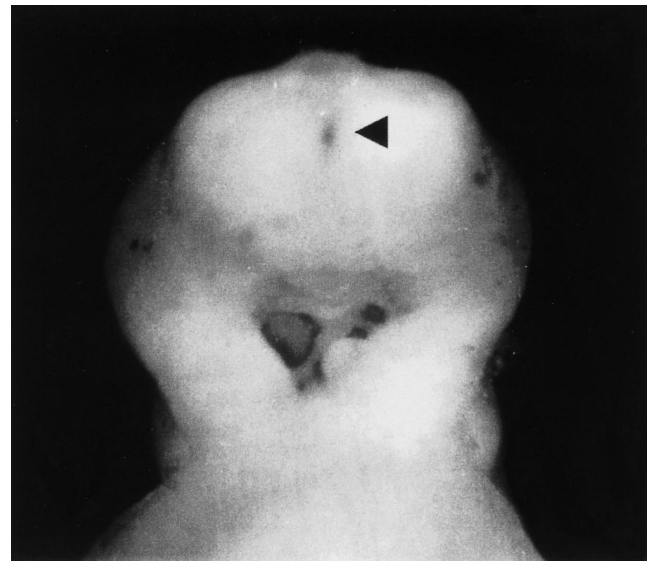


Fig. 4 Frontal view of the head of an embryo at CS11 (no. 31163). The anterior neuropore (*arrowhead*) is closing near the rostral end of the prosencephalon

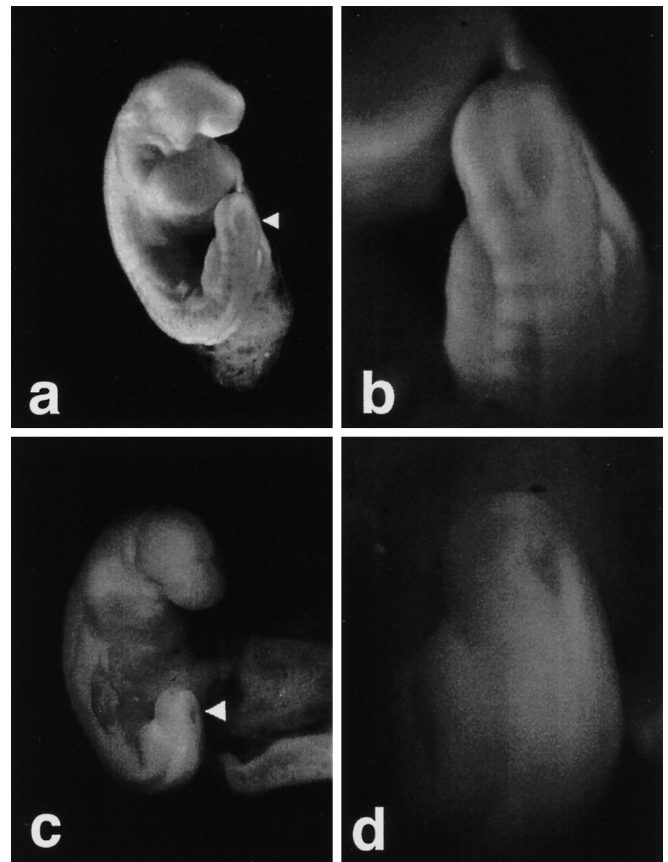
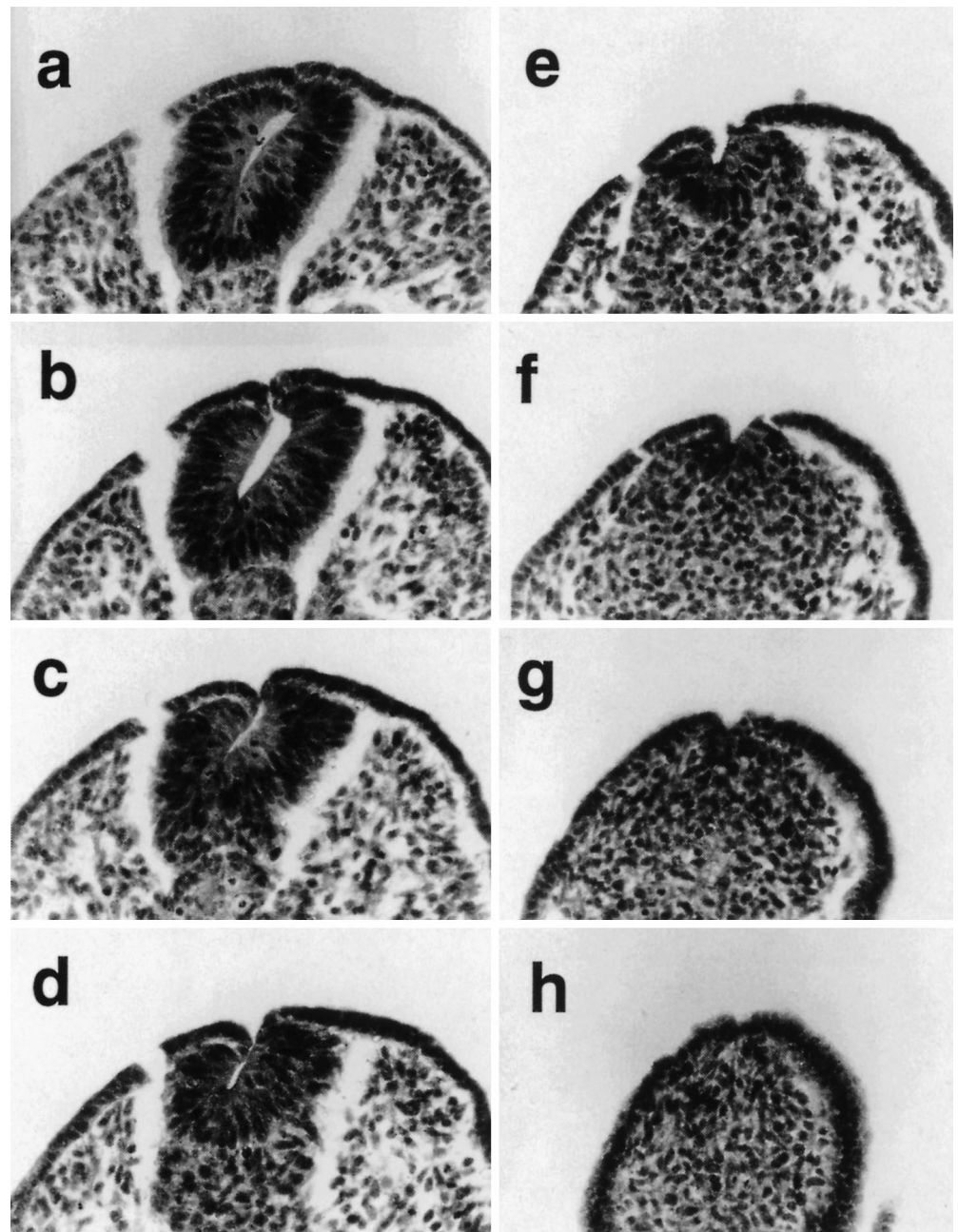


Fig. 5 Human embryos at CS12 with the closing posterior neuropore (a no. 32618; c no. 33006) and the close-up views of their neuropores (b and d, respectively). *Arrowheads* indicate the posterior neuropores. Note that the posterior neuropores appear pear-shaped, with cranial tapered and caudal wide portions. The floor of the neural groove appears to become shallower toward its caudal end and continuous with the surface ectoderm of the tail bud

Fig. 6 Serial transverse sections of a CS12 embryo (no. 589) through the closing posterior neuropore in a rostro-caudal sequence (a–h). The neural folds are fused with each other in **a**, but the neuropore is still open in **b–g**. Toward the caudal end of the posterior neuropore, the neural groove became gradually shallower and the floor of the neural groove appeared to be continuous with the surface ectoderm of the tail bud (**h**). The boundary between the neuroepithelium and the surrounding mesenchyme also became less distinct toward the caudal end of the neural groove (**e–h**)



dally, forming the dorsal and terminal lips of the anterior neuropore, respectively.

Meanwhile the caudal extension of the closure from Site A proceeds all the way down to the lumbar level, and finally forms the posterior neuropore near or at the caudal end of the neural groove. To observe the closure of the posterior neuropore in detail, we examined 30 embryos at CS12 under a dissection microscope. In 8 embryos (20–23 somite pairs) the posterior neuropore was widely open below the lumbar level. The posterior neuropore was small and about to close in 5 cases (23–26 somite pairs). In the remaining 17 cases (26–29 somite pairs), the posterior neuropore had already been closed. In the 5 cases at the final stage of NT closure, the poste-

rior neuropore appeared pear-shaped, with cranial tapered and caudal wide portions (Fig. 5). The floor of the neural groove appeared to become shallower toward its caudal end, and the boundary between the surface ectoderm and the caudal end of the neural groove was not distinctly demarcated.

We examined histologically 18 embryos at CS12. Five of them had a widely open posterior neuropore. In 12 cases, the posterior neuropore was already closed. In the remaining case, the posterior neuropore was small and appeared to be disappearing. Figure 6 shows serial transverse sections through the posterior neuropore of the embryo at the final stage of neurulation (embryo No. 589). Toward the caudal end of the poste-

pore, the neural groove became gradually shallower and the floor of the neural groove appeared to become continuous with the surface ectoderm at its caudal end. The boundary between the neuroepithelium and the surrounding mesenchyme also became less distinct toward the caudal end of the neural groove. As shown in Figs. 5 and 6, showing the closing posterior neuropore, the fusion of the neural folds seem to proceed from the cranial tapered portion toward the caudal end of the neuropore, where the caudal NT closure is completed. Thus it is likely that the closure of the posterior neuropore in human embryos is unidirectional and the closure of the human NT neither initiates at its caudal end nor proceeds rostrally from the caudal end.

As described above, our direct observation of human embryos with the closing NT demonstrated that NT closure in the human most likely initiates at multiple sites, contrary to the traditional “zipper” or continuous closure model. In addition to the first closure starting at the prospective cervical region (Site A), the second closure of the NT initiates at the boundary between the mesencephalon and rhombencephalon (Site B). Since the closure from Site A and that from Site B were observed simultaneously only in 3 embryos with 10 somite pairs in the present series and since the opening between the two closures is small, it is likely that the opening between the two closures disappears quickly during the later half of CS10 and can be observed only briefly. Therefore, the opening between the first and second closures could have been overlooked by previous investigators. A careful review of previous publications in which early human embryos, mainly from the Carnegie Collection, were drawn in detail, supports the finding that the second initiation site of NT closure occurs around the level of the mesencephalic-rhombencephalic boundary (Corner 1929; Heuser and Corner 1957; Müller and O’Rahilly 1985; O’Rahilly and Müller 1987).

Next, the third closure initiates at the rostral tip of the neural groove (Site C) and proceeds caudally. However, the caudal extension of this closure is limited and it meets the cranial extension of the closure from Site B near the rostral end of the prosencephalon to close the anterior neuropore. On the other hand, the caudal extension of the closure from Site A proceeds to the caudal end of the neural groove where the posterior neuropore closes. Therefore, the closure of the anterior neuropore is bidirectional, whereas that of the posterior neuropore is unidirectional in human embryos.

Model for NT closure in the human embryo

Based on the observation of human embryos with a fusing NT (CS 10–12), we propose the following model for NT closure in human embryos (Fig. 7). NT closure commences at the level of the future cervical region, which is generally accepted as the initiation site of NT closure in humans (Site A). The closure from this point proceeds both rostrally and caudally. Neural folds begin to fuse at the second initiation site around the mesencephalic-

rhombencephalic boundary (Site B), and the small opening between the first and second closures disappears quickly.

The NT begins to close also from the rostral end of the neural groove (Site C). The closure from Site C proceeds caudally for a short distance over the anterior part of the prosencephalon where this closure meets the cranial extension of the closure from Site B to close the anterior neuropore. The closure of the anterior neuropore is therefore bidirectional and is completed at the anterior part of the prosencephalon.

On the other hand, the caudal extension of the first closure from Site A proceeds all the way down to the caudal end of the neural groove, where the posterior neuropore is formed. The closure of the posterior neuropore is unidirectional and never proceeds cranially from its caudal end.

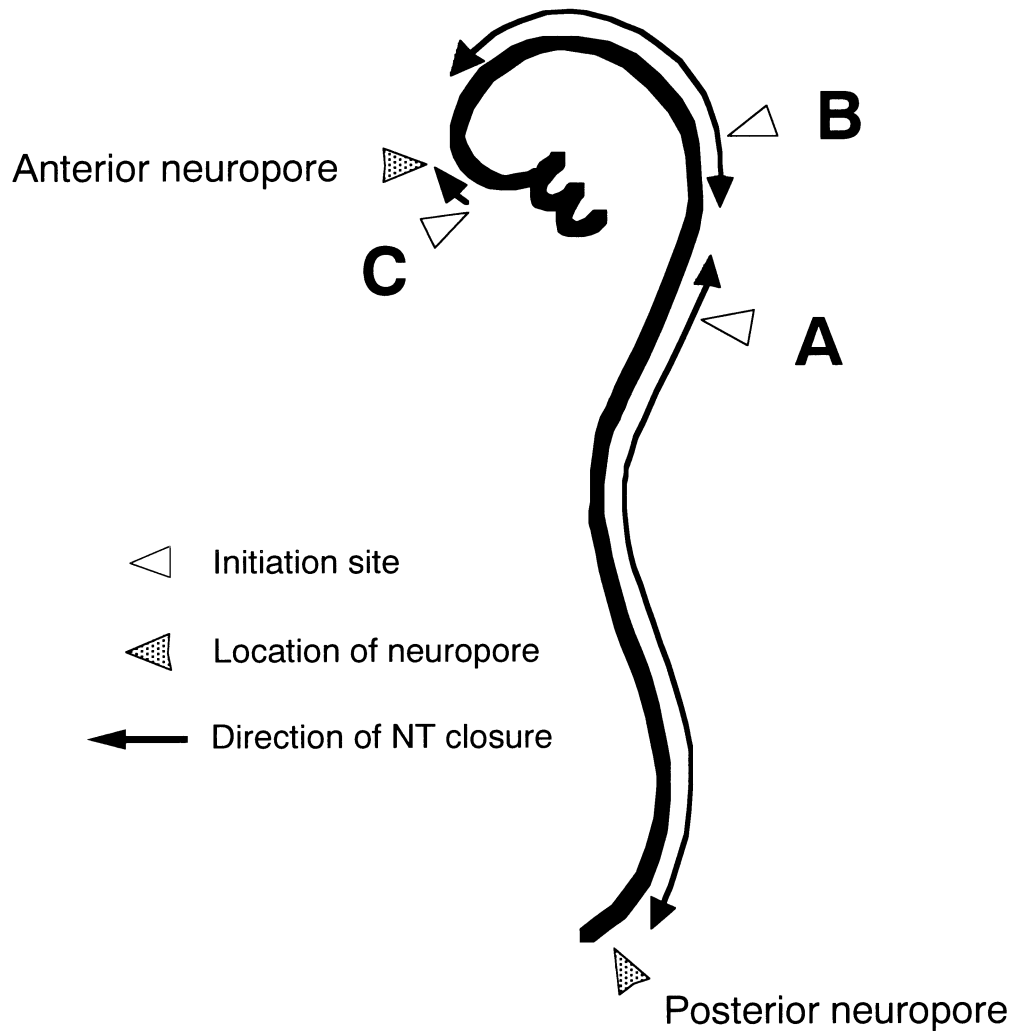
This model of NT closure in humans is different not only from the traditionally “zipper” or continuous closure model of the human NT but also from the multi-site closure model proposed by van Allen et al. (1993). Van Allen et al. (1993) simply extrapolated the mouse data to the human and suggested that the second closure (their “closure 2”) initiates at the junction between the prosencephalon and mesencephalon. In our study, however, NT closure was never observed to initiate there but the second closure was found to take place at the mesencephalic-rhombencephalic boundary. Van Allen et al. (1993) also postulated that a closure initiates at the caudal end of the neural groove and spreads cranially (their “closure 5”). However, we found that the closure of the posterior neuropore does not initiate at its caudal end but the caudal extension of the closure from Site A reaches the caudal end of the neural groove where the posterior neuropore closes. It seems that the mode of NT closure differs between different animal species, and this will be discussed further in the following section.

Although several authors have reported that NT closure in rodent embryos initiates at multiple sites, the applicability of the intermittent or multi-site NT closure model to the human was never argued before the report by van Allen et al. (1993) probably for the following reasons. First, early human embryos at the neurulation stage are only rarely available, probably because neurulation is a relatively rapid event and can be observed only briefly at late CS10 and CS11. In addition, a number of human embryos collected to date were derived from spontaneous abortion or maternal death, therefore maceration and other postmortem changes prevented the preservation of their normal morphology. The Kyoto Collection of Human Embryos includes a substantially large number of early human embryos that were procured after termination of pregnancy in healthy women and this has enabled us to observe as many as 68 embryos at the neurulation stage.

Species differences in the mode of NT closure

Previous studies have revealed that there is a considerable variation in the mode of neurulation among differ-

Fig. 7 Model for multi-site NT closure in the human embryo. *Open triangles* show three initiation sites (*A* the cervical level, *B* the mesencephalic-rhombencephalic boundary, and *C* the rostral tip of the neural groove) and *arrows* indicate the directions of NT closure. *Dotted triangles* show the sites of closure of the anterior and posterior neuropores



ent animal species and even among different strains of the same species. The mouse is one of the species most extensively studied, and separate initiation sites for cranial NT closure have been demonstrated (Golden and Chernoff 1983, 1993; Juriloff et al. 1991; Tom et al. 1991). The first contact and fusion of apposed neural folds occurs at the presumptive boundary between the rhombencephalon and the spinal cord, which is similar to the event observed in human embryos, and this closure proceeds bidirectionally both rostrally and caudally. The second closure initiates at the junction between the prosencephalon and mesencephalon, and also proceeds bidirectionally. Therefore, in mouse embryos at some stage of neurulation, two prominent openings are recognized simultaneously – over the prosencephalon (rostral to the second closure) and over the mesencephalon-rhombencephalon (between the first and second closures; Juriloff et al. 1991). However, the site of initiation of the second closure may be different in some strains of mice. In the SWV/Bc strain, the second initiation site is located further rostrally in the prosencephalic region, rather than at the prosencephalon-mesencephalon junction (Juriloff et al. 1991).

In the rabbit embryo, Peeters et al. (1998a) observed an initiation site of NT closure at the level of the mesencephalic-rhombencephalic transition as in humans, but other initiation sites also take place at the prosencephalic-mesencephalic boundary and at the level of the first pairs of somites. Van Straaten et al. (1996) reported a detailed description of NT closure in the chick embryo and demonstrated that the first closure of the NT occurs in the future mesencephalon, and the second closure is seen at the rhombocervical level in the form of multiple contacts of neural folds. Multi-site closure of the NT has been observed also in the rat (Christie 1964; Edwards 1968) and hamster (Keyzer 1972; Shenefelt 1972).

Therefore, multi-site closure of the NT may be a phenomenon universally observed in a variety of animal species, but the process and initiation sites of NT closure appear to be not the same between the human and many other animal species. For example, the mode of NT closure is different in the human and mouse embryos in the following points. The second closure of the NT initiates at the mesencephalic-rhombencephalic boundary in the human, while it occurs at the prosencephalic-mesencephalic junction in many strains of mice. Subse-

quently two separate large openings exist simultaneously, over the prosencephalon and mesencephalon, in the mouse embryo at a certain stage of neurulation, but such separate openings of the NT over the prosencephalon and mesencephalon are never observed in the human. In the human embryo, after the first and second closures meet over the rhombencephalon, the closure from Site B proceeds rostrally over the mesencephalon and prosencephalon and meets the closure extending from the rostral end of the NT (Site C) at the anterior part of the prosencephalon.

Although the closure of the anterior neuropore has been rather extensively examined, that of the posterior neuropore has been studied relatively poorly both in the human and laboratory animals. One exception was the study by Sakai (1989) who observed the closure of the posterior neuropore in embryos of the ICR strain mouse and reported that its closure occurs at what was originally the caudal end of the neural plate and proceeds rostrally to meet the caudal extension of the closure initiating at the cervical region. He concluded that the closure of the posterior neuropore completes at the future sacrococcygeal level of the spinal cord, which was not the caudal end of the neural plate. On the other hand, in the rabbit embryo, the posterior neuropore is at first long and slender, with tapered cranial and wide caudal portions (Peeters et al. 1998a). The groove closes in a cranio-caudal sequence and the posterior neuropore disappears at the caudal end of the neural plate. The mode of closure of the posterior neuropore in the chick resembles that of the rabbit embryo (van Straaten et al. 1996). Therefore, the mode and sequence of closure of the posterior neuropore in the human embryo appear similar to those observed in the rabbit and chick embryos.

Recently it was revealed by Peeters et al. (1998b) that the axial curvature of the embryo is an important factor in neurulation and that the rate of NT closure increases as the axial curvature of the embryo decreases, which can be a cause of the species-specific relationship between NT closure and axial curvature. They found that human and rabbit embryos are flat at younger stages and demonstrate a similarly high rate of NT closure, which is different from the corresponding data for the mouse and rat.

The mode of NT closure may be so different among species that care should be taken when extrapolating embryological and teratological data in laboratory animals to the human. The multi-site closure model for the human NT proposed by van Allen et al. (1993) and some other papers that followed (Golden and Chernoff 1995; Seller 1995a) provided a new insight into the mechanisms of neurulation in human embryos, but simply extrapolating mouse data to the human may sometimes be misleading.

Human embryos with cranial NTD

Among well-preserved embryos in the Embryo Collection, there were 47 cases at CS11–23 that had an open

lesion of the NT at the cranial and/or cervical regions. Ten cases were obtained following spontaneous abortion and 14 cases were terminated following maternal signs of threatened abortion. The remaining cases were obtained after induced abortion given to women during uncomplicated pregnancy. Of these NTD embryos, 23 cases (49%) had signs of intrauterine death (maceration).

Forty-seven embryos with cranial NTD were classified into the following six groups according to the location of the NTD (Fig. 8):

Type I The NT is open at the frontal part of the head (prosencephalon or telencephalon).

Type II The NT is open at the parietal region of the head (prosencephalon and mesencephalon).

Type III The NT is open over the fourth ventricle (rhombencephalon).

Type IV The NT is open at the mesencephalon through rhombencephalon (occasionally including the neck). The open lesion includes the fourth ventricle.

Type V The NT is open from the frontal part of the head through the cervical region.

Type VI Total dysraphism, involving the entire length of the brain and spinal cord.

If we apply the proposed model of NT closure described above, the pathogenesis of each type of cranial NTD can be explained as follows:

Type I The closure from Site B and that from Site C failed to meet each other over the prosencephalon, probably because the closure from Site B did not move sufficiently toward the anterior part of the prosencephalon.

Type II The closure from Site B failed to extend anteriorly over the prosencephalon, and the closure from Site C moved caudally to some extent to compensate the former closure but failed to meet it.

Type III The closure from Site A and that from Site B failed to meet over the rhombencephalon (fourth ventricle). This defect may be small initially, but the neural tissue overgrows through the open lesion. The closure of the other parts of the NT was not affected.

Type IV The primary defect may be a lack of the closure that should initiate at Site B. In compensation, the closure from Site C may proceed caudally to the parietal region.

Type V It appears that the closure from site B failed to initiate, and in addition, closure from Site C also failed to move caudally to compensate it.

Type VI Neither the closure from Site A nor that from Site B were formed, and the closure from Site C moved little, if any.

The location of the NTD in the 47 embryos are schematically shown in Fig. 9. Some characteristic features were noted in the distribution of each type of NTD. Embryos with total dysraphism (Type VI), the most severe type of NTD, were young and never encountered after CS15, suggesting that embryos with such serious NTD die rather early in utero and are eliminated. About two-thirds (17/25) of embryos with NTD at the rhombencephalon

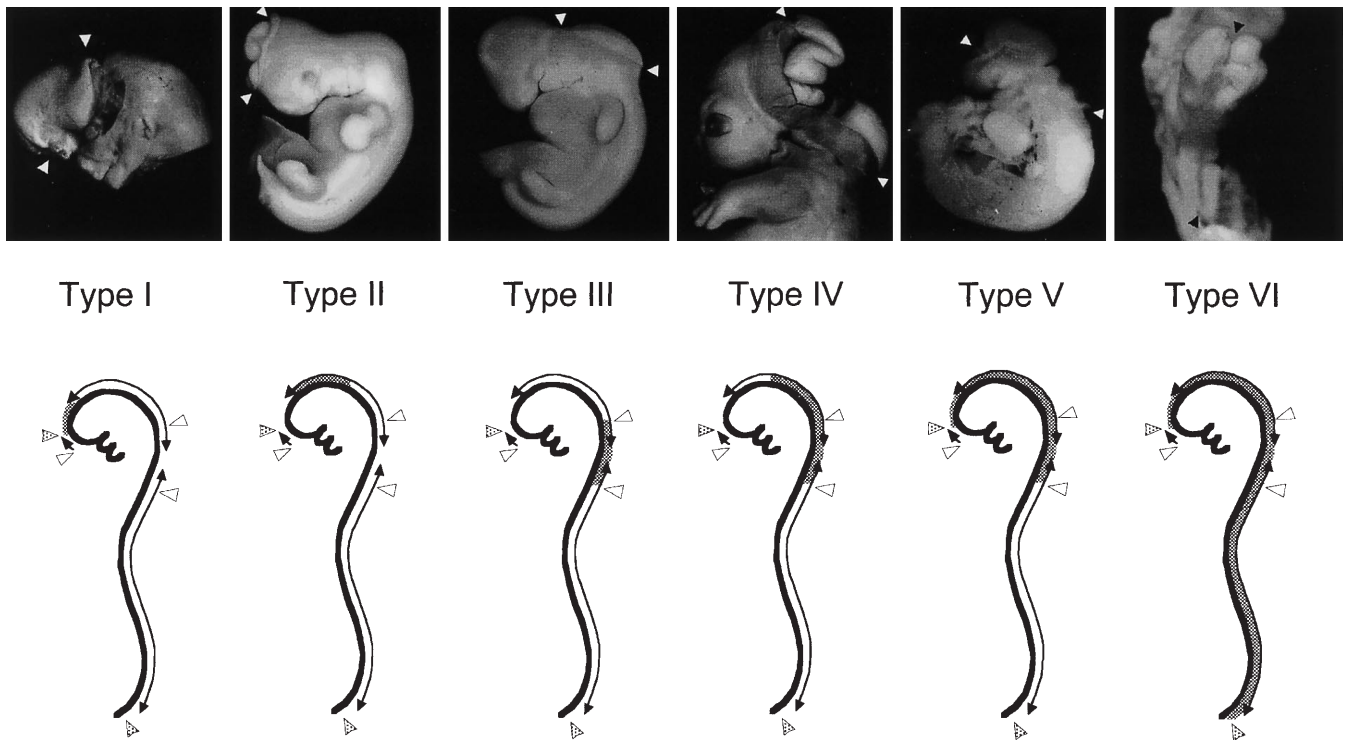


Fig. 8 Classification of cranial NTD in human embryos by the location of the defect. Representative cases of each type of NTD are shown in the upper row of the figure. *Arrowheads* in the embryo pictures indicate the range of the open lesion. In the schematic drawings in the lower row, shaded areas indicate the sites of NTD. *Open triangles* show the initiation sites of NT closure, and *dotted triangles* the anterior and posterior neuropores, just as in **Fig. 7**: *type I* The NT is open at the frontal part of the head (no. 10501, CS23); *type II* the NT is open at the parietal region of the head (no. 11299, CS16); *type III* the NT is open over the fourth ventricle (no. 10494, CS15); *type IV* the NT is open over the mesencephalon through rhombencephalon (no. 7176, CS20); *type V* the NT is open from the frontal part of the head through the cervical region (no. 21916, CS14); *type VI* total dysraphism, involving the entire length of the brain and spinal cord (No. 21924, CS11)

or the fourth ventricle (Types III, IV and V) were younger than CS19, while over 70% (10/14) of the cases with NTD over the prosencephalon and/or mesencephalon only (not involving the rhombencephalon and spinal cord; Types I and II) were older than CS20. Thus the location and severity of the NTD surely affects the intrauterine survival of NTD embryos. It is likely that the involvement of the rhombencephalon (future brain stem) significantly affects the intrauterine survival of human embryos with NTD.

Human embryos with caudal NTD

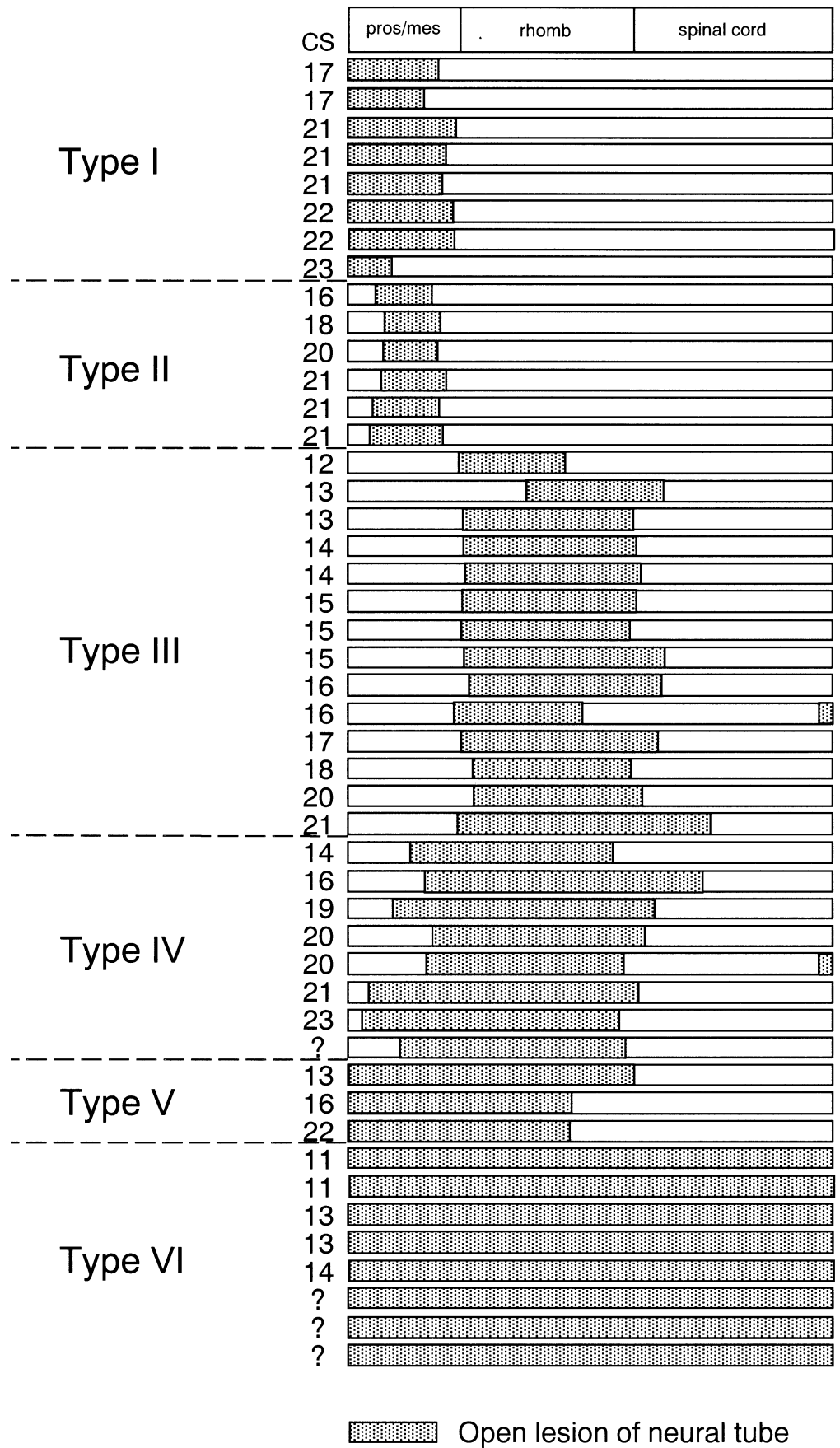
There were 61 cases at CS11–23 that had an open lesion of the NT at the lumbosacral level. The distribution of the location of the caudal NTD is shown in Fig. 10. Ten of these cases were associated with cranial NTD also (Fig. 9). Twenty-nine cases were obtained after sponta-

neous or threatened abortion. The remaining cases were procured after induced abortion given during uncomplicated pregnancy. Thirty-four cases of these NTD embryos (56%) had a sign of intrauterine death (maceration). The rostral end of the caudal NTD was variable among the cases. In 10 of the cases, the NT was patent continuously from the thoracic through the sacral levels (8 of them were of total dysraphism). In another 28 cases, the anterior end of NTD was at the lumbar level and more cranial to the posterior border of the lower limb bud. In the remaining 23 cases, the NT was open within the sacral levels (below the posterior border of the lower limb). Although the rostral end of the NTD was quite variable, the caudal end of the defect in our NTD embryos was always at the level of the 30th or 31st somite segment, which corresponds to the caudal end of the neural groove. Only two cases at CS14 and CS16 were exceptional in that the NT was open to the tip of the tail bud. If we apply our model of NT closure, caudal NTD (lumbosacral myeloschisis) can be explained as a result of the failure of the closure from Site A to reach the caudal end of the neural groove.

The distribution of embryos with caudal NTD by developmental stage was similar to that of the total embryo population (Matsunaga and Shiota 1977; Shiota 1991), indicating that the type of NTD at the lumbosacral region may not significantly affect the intrauterine survival of the abnormal embryo, at least during the embryonic period proper.

Van Allen (1993), Golden and Chernoff (1995) and Seller (1995b) claimed that the pathogenesis of human NTD can be conveniently explained by their multi-site NT closure model. However, the majority of NTD cases can be more logically explained by our new model of human NT closure as described above.

Fig. 9 The distribution of embryos with cranial NTD by the defect type and developmental stage (CS). The NT is expressed as a straight bar. *Dotted areas* indicate the locations of NTD



- O'Rahilly R, Müller F (1987) Developmental stages in human embryos. *Carnegie Ins Washington Publ* 637
- O'Rahilly R, Müller F (1994) Neurulation in the normal human embryo. Neural tube defects. *Ciba Found Symp* 181 :70–89
- Padmanabhan R, Naruse I, Shiota K (1999) Caudal dysgenesis in staged human embryos (Carnegie stages 16–23). *Am J Med Genet* 87: 115–127
- Peeters MCE, Viebahn C, Hekking JWM, Straaten HWM van (1998a) Neurulation in the rabbit embryo. *Anat Embryol* 197: 167–175
- Peeters MCE, Hekking JWM, Shiota K, Drukker J, Straaten HWM van (1998b) Differences in axial curvature correlate with species-specific rate of neural tube closure in embryos of chick, rabbit, mouse, rat and human. *Anat Embryol* 198: 185–194
- Sakai Y (1989) Neurulation in the mouse: manner and timing of neural tube closure. *Anat Rec* 223: 194–203
- Schoenwolf GC, Smith JL (1990) Mechanisms of neurulation: traditional viewpoint and recent advances. *Development* 109: 243–270
- Seller MJ (1995a) Further evidence for an intermittent pattern of neural tube closure in humans. *J Med Genet* 32: 205–207
- Seller MJ (1995b) Sex, neural tube defects, and multisite closure of the human neural tube. *J Med Genet* 58: 332–336
- Shenefelt RE (1972) Morphogenesis of malformations in hamsters caused by retinoic acid: relation to dose and stage of treatment. *Teratology* 5: 103–118
- Shiota K (1991) Development and intrauterine fate of normal and abnormal human conceptuses. *Congenital Anomalies* 31: 67–80
- Straaten HWM van, Janssen HCJP, Peeters MCE, Copp AJ, Hekking JWM (1996) Neural tube closure in the chick embryo is multiphasic. *Dev Dyn* 207: 309–318
- Straaten HWM van, Peeters MCE, Szpak KF, Hekking JWK (1997) Initial closure of the mesencephalic neural groove in the chick embryo involves a releasing zipping-up mechanism. *Dev Dyn* 209: 333–341
- Tom C, Juriloff DM, Harris MJ (1991) Studies of the effect of retinoic acid on anterior neural tube closure in mice genetically liable to exencephaly. *Teratology* 43: 27–40