Neural tube closure defects: a discussion of current models and clinical presentation of a skull from the Museum of Anatomy of a Brazilian University

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SUMMARY

Currently, neural tube defects (NTD) have an incidence rate of 1/1000 of those born alive. Their occurrence relates to the primary neurulation period. In this context, there are two main embryogenesis models that attempt to explain neural tube closure: the continuous closure model and the multisite closure model. In this work, we studied a skull with NTD from the collection of the Federal University of São Paulo, Paulist School of Medicine, where the cephalic perimeter and index were measured and the dental arcade was used to estimate chronological age. From this analysis we conclude that this was a microbrachycephalic skull, with anterior fontanelle disjunction and a neural tube closure defect at site 2, according to the multisite classification model. In general, NTDs present a high degree of clinical and etiologic heterogeneity. The causes for this broad diversity of defects are the intrauterine involvement of teratogenic factors, among them maternal alcoholism, use of carbamazepine during pregnancy, and folic acid deficiency, among others. There are many genes potentially involved in this class of defects configuring as multifactorial. Thus, the multisite closure model allows one to explain many of these defects by suggesting the possibility of a different genetic control for each closure site, together with different sensitivities to environmental factors, both interacting with each other as a probable NTD etiology.

Key words: Neural tube defects – Skull – Microcephaly – Cranial sutures

INTRODUCTION

In recent years, despite the extensive clinical and epidemiological studies that have been carried out and little is yet known about the factors that determine neural tube defects (NTDs). In humans, neural tube (NT), the embryologic precursor of the brain and spinal cord, is formed by the mid-line fusion of the bilateral neural folds during the fourth week

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of gestation (Nakatsu et al., 2000; Chen, 2006; Jones, 2006).

Currently, there are two main models that attempt to explain neural tube closure: the first is the continuous closure model and the other is the multisite closure model. In the first model, NT closure in human embryos is described as a process that begins in the cervical region (approximately between the third and sixth somite pairs) and continues bilaterally to the rostral and caudal end of the neural plate, similar to a zipper (Figure 1) (Heuser and Streeter, 1941; Harmen and Prickett, 1942; Graves, 1945; Hamburger and Hamilton, 1951; Edwards, 1968; Davignon et al., 1980). Closure is complete when the anterior and posterior neuropores are closed at around 24 and 28 days, respectively, after fertilization (O'Rahilly and Müller, 1994). Thus, when the anterior neuropore fails to close it results in NTDs such as anencephaly and encephalocele; if the posterior neuropore fails to close, it results in meningocele, myelomeningocele (or meningomyelocele) and spina bifida.

Questioning this single-site closure model, in their study of clinical cases of NTDs in human embryos Van Allen et al. (1993) suggested a model consisting of many NT closure sites, based on previous experimental studies in animals (Golden and Chernoff, 1983; MacDonald et al., 1989; Tom et al., 1991; Juriloff et al., 1991; Golden and Chernoff, 1993). In this model, Van Allen et al. (1993) proposed the existence of 5 closure sites in humans. Site 1 would start between somites 2-4, at the presumed limit between the spinal cord and the myelencephalon, and would proceed bidirectionally. Fusing caudally, site 1 would form the thoracic neural tube (the medulla) and the posterior neuropore. Rostrally, it would proceed slightly beyond the otic pits (the inferior aspect of the rhombencephalon). Site 2 would begin at the junction between the prosencephalon and the mesencephalon. This site would also proceed bidirectionally, forming two cranial neuropores, one in the prosencephalon region and the other in the mesencephalon region. Caudally, site 2 would progress over the mesencephalon to end at the upper point of the rhombencephalon. Rostrally, it would proceed over the prosencephalon to meet site 3. Concurrent with site 2, site 3 would begin in the rostral-most portion of the neural fold, adjacent to the stomodeum, proceeding caudally to meet site 2, closing the prosencephalic neuropore (anterior neuropore),

this junction being located in the interorbital region of the face. The most rostral end of the neural plate would correspond to the position of the upper lip. Site 4 would begin on the caudal end of the rhombencephalon and would be unidirectional, progressing rostrally to meet site 2. In sites 1-3, fusion would occur following the elongation of the lateral limit of the neural plate and elevation of the fold (Gordon, 1985; Jacobson e Tam, 1982). The fold
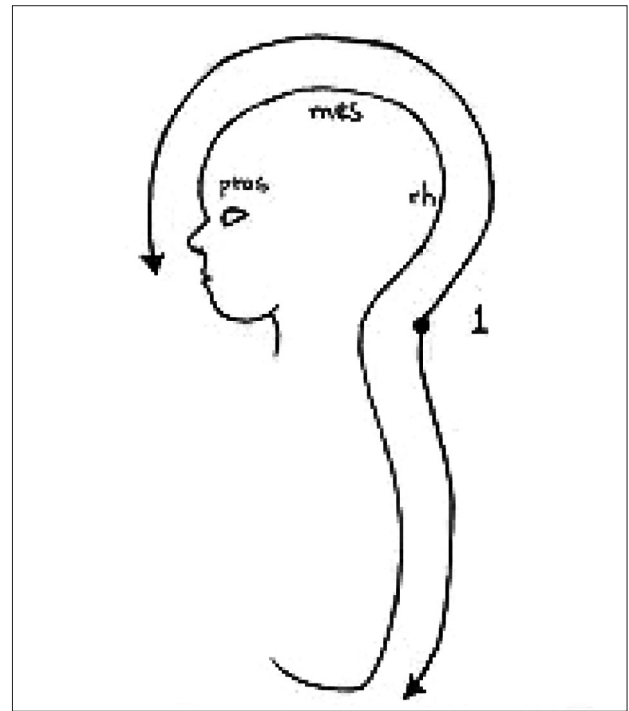

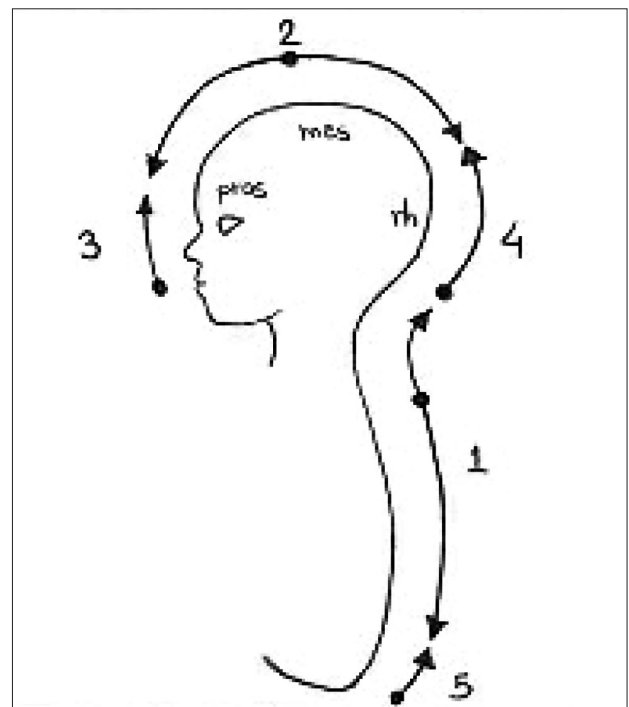**Figure 1.-** Single-site NT closure model.
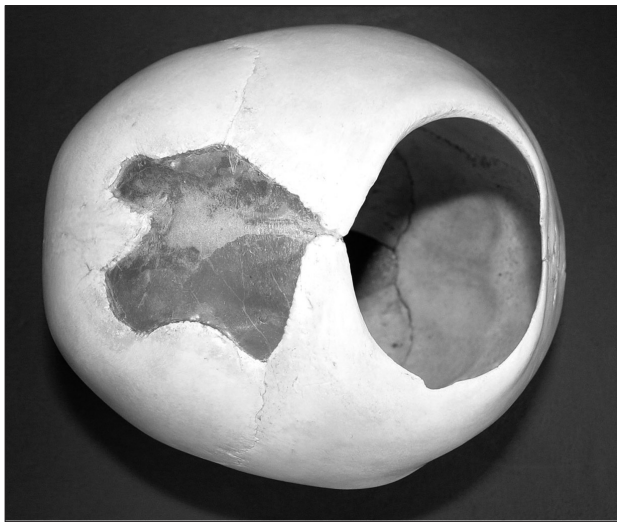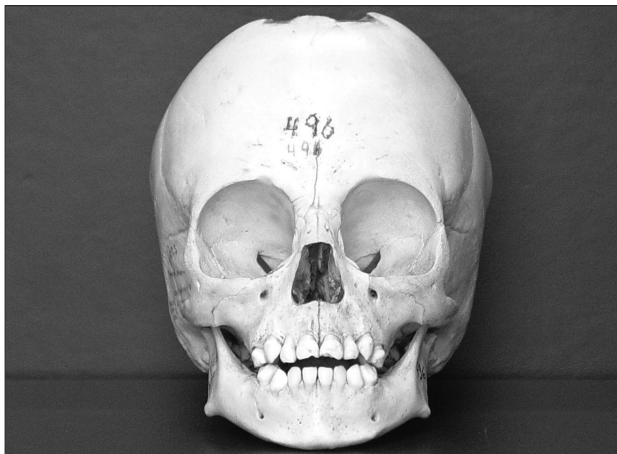

Figure 2.- Multi-site NT closure model.

Figure 3.- Superior view of the skull.

Figure 4.- Anterior view of the skull showing the dental arcade with primary dentition.

elevation process is complex and influenced by the cytoskeleton and the shape of neuroepithelial cells, mesenchymal cells and the extracellular matrix. Site 4 is unique, since its folds remain separated and closure occurs by growth of a membrane that covers the rhombencephalon. Finally, site 5 is the most caudal one, located in the lumbosacral region (between L2-S2) and its closure progresses rostrally to meet site 1, forming the posterior neuropore. Currently, the existence of site 5 in humans is under debate and it has been proposed that site 1 would be responsible for the formation of the caudal neuropore (posterior), limiting the total number of sites to 4 (Figure 2) (Nakatsu et al., 2000).

Using the work by Van Allen et al. (1993) as a reference, here we discuss the anatomical characteristics and suggest possible etiologic agents involved in an NTD observed in one of the skulls of the collection of the Federal University of São Paulo – Paulist Medical School.

MATERIALS AND METHODS

Piece number 496 of the collection of articulated skulls from the Museum of Skulls, Division of Descriptive and Topographic Anatomy, Department of Morphology and Genetics of the Federal University of São Paulo – Paulist Medical School - was studied. This collection was begun in 1951 and currently consists of 510 skulls grouped by age, race and sex.

For the analysis of this skull, the cephalic perimeter and index were measured and chronological age was estimated by analyzing the dental arcade.

The cephalic perimeter was measured using a metric tape passing above the glabella and over the external occipital protuberance. The value obtained was associated with specific population lines (Hall et al., 1989). The cephalic index was calculated from the relationship between the maximum cross sectional (CSD) and anteroposterior diameters (APD) of the skull obtained with an extension compass and expressed in percentages: ultra-dolichocephalic skull (<64.9%), hyperdolichocephalic skull (65.0% to 69.9%), dolichocephalic skull (70.0% to 74.9%), mesencephalic skull (75.0 to 79.9%), brachycephalic skull (80.0 to 84.9%), hyperbrachycephalic skull (85.0 to 89.9%), and ultrabrachycephalic skull (>90%) (Hall et al., 1989; Lemire, 2000; Di Dio, 2002).

Digital capture was used for the photographic records, using a Sony MVC-CD400™ digital camera.

RESULTS

The skull (dated from the year 1970 and belonging to a male child) presents a circular opening in the parietal region with an approximate diameter of 5.0 cm in the anteroposterior axis and a diameter of 5.4 cm in the laterolateral axis with regular and smooth borders (Figure 3). The medical record of the piece, in the archives of the Museum of Skulls, and the symmetric and perfect architecture of the borders confirms that there was indeed a primary malformation in the NT closure.

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The medical record provides a chronological age of 1 year. However, the dental arcade has a primary dentition with incisors, canines and first upper and lower molars, bilaterally (Figure 4). This arrangement suggests that the chronological age of the skull would be between 16 and 18 months (Di Dio, 2002).

The cephalic perimeter obtained was 40 cm. This value, when placed in population curves within the age group ranging from 16 to 18 months, is below the second negative standard deviation and therefore reveals a microcephalic skull.

The calculated cephalic index was 82.9% and this represents a brachycephalic pattern (APD of 13.20 cm and CSD of 10.95 cm). The anterior fontanelle has an average diameter of 3.5 cm, which is above the second standard deviation for the estimated age range, therefore showing a disjunction of the anterior fontanelle (Figure 3).

External morphological analysis revealed a parallel asymmetry between the coronal sutures and a deviation of the frontal suture median line (Figures 3 and 4).

It may therefore be concluded that this is a microbrachycephalic skull with a disjunction of the anterior fontanelle and a neural tube closure defect at site 2, according to the classification by Van Allen et al. (1993).

DISCUSSION

Neurulation, a morphogenetic event of great importance in human development, constitutes a complex process that occurs in two phases in the embryos of mammals. In normal human embryos, primary neurulation is characterized by the neural furrow and folds, becoming apparent 18 days after fertilization. On the 20th day, the three main divisions of the brain - the prosencephalon, mesencephalon and rhombencephalon - can be distinguished while the neural furrow is still open. On the 22nd day, the neural folds begin to fuse close to the junction between the brain and the spinal cord, as the cells of the neural crest are arising in the neuroectoderm. The fusion of the rostral NT occurs rapidly within a few hours when the embryo is around 24 days. The closure of the rostral neuropore has been shown to be bidirectional and can occur simultaneously in many areas. The caudal neuropore closes at around 26 days and the final closure level is located approximately in the future somite pair 31,

corresponding to the 2nd sacral vertebra (S2). By 28 days, the NT is completely closed in normal human embryos. Secondary neurulation begins on the 28th day at the level of somite pair 31 (S2) and is characterized by the differentiation of the eminence cells along the neural cord by cavitation (Van Allen et al., 1993; Sadler, 2004).

In this work, we studied an NTD in a skull from the collection of the Federal University of São Paulo – Paulist Medical School. The incidence of NTDs at birth varies. In Ireland it is close to 1% and in the USA, roughly 0.2%, and two-thirds of the children affected are female (Nussbaum et al., 2001). The interaction between genetic and environmental factors is accepted as the cause for multifactorial NTDs. The geographical variation of NTDs is associated with ethnic factors (genetic as well as environmental and/or cultural) and the variation in time with environmental, fundamentally socioeconomic, factors (Calvo and Martinez-Frias, 2002). Among the disorders associated with NTDs (Table 1), teratogenic embryofetopathies stand out, determined by the use of alcohol, aminopterin and carbamazepine, as well as a history of gestational diabetes and deficiency of nutritional factors associated with folate (Schüler et al., 1998; Sanseverino et al., 2001).

Table 1. Known etiologies with neural tube closure site specificity (adapted from Van Allen et al., 1993).

Disorders associated with NTD	Common closure sites
- Nutritional factors folate deficiency	2, 4, 1
- Teratogen embryofetopathies	
• Alcohol	2,1
• Aminopterin	anencephaly, cephalocele
• Carbamezapine	2, caudal 1
• Maternal diabetes	2, caudal 1, 5, canalization, caudal dysgenesis sequence

The adverse effects of using alcohol during pregnancy are widely recognized and there are references to such effects dating from antiquity. The study of Lemoine et al., in 1968 provided a chart of facial dysmorphisms and psychomotor disturbances of 25 children born to alcoholic women and, from that point, research into alcohol as a teratogenic agent began. Thus, the term fetal alcohol syndrome (FAS) was introduced to define the set of characteristics associated with maternal alcoholism, constituted by malformations, dysmorphisms, especially facial, growth retardation, retardation of psychomotor development and diminished intellectual development (Sanseverino et al., 2001). Intrauterine expo-

sure to ethanol is estimated as moderate to high if the pregnant woman is an assiduous consumer; that is, she drinks more than six units of wine or beer per day. In this group of pregnant women, the central nervous system (CNS) anomalies generally found in their off-spring are: diminished cephalic perimeter at birth, and structural brain anomalies or neuro-logical signs, such as decreased fine motor con-trol, among others. Taking into account that the skull studied here has the morphological characteristics of microbrachycephaly, anterior fontanelle disjunction and neural tube closure defect at site 2, it may be assumed that there were teratogenic factors involved during preg-nancy and that this child was subject to intrauterine ethanol exposure. Evidently, we cannot discard the hypotheses of an isolated primary malformation of the CNS or of a mal-formation syndrome where, besides the cranial facial dysmorphisms, there are other deviations of the external morphological phenotype. It is impossible to prove such assertions since we do not have the remaining axial and appendicular anatomy of the skull studied.

Currently, carbamazepine, a drug used in patients with epilepsy, especially during grand mal seizures, is associated with a risk of roughly 1% for NTDs, such as meningomye-locele (Schüler et al., 1998).

Another known etiology for NTDs is the presence of gestational diabetes. The rate of fetal malformations in type I diabetes mellitus is 7.5-12.9%, in which heart and neural tube defects are the most frequent. The use of folic acid (vitamin B_9 or M) is essential to prevent NTDs, whose risk is of 1% in women with type I diabetes mellitus. Folate acts as a cofac-tor for an enzyme involved in the biosynthesis of DNA and RNA and is also a supplier of methyl groups to methylation cycles. Folate deficiency leads to an up-regulation of folate receptors, which are ubiquitous, and mediates folate uptake in physiological levels. Researchers are currently studying a number of different gene products involved in the metabolism of folic acid, among them methyl-enetetrahydrofolate reductase (MTHFR), a cystathionine β-synthase. Recent studies have suggested that homozygotes for the C677T mutation, a thermolabile variant of the MTHFR gene, would have an increased risk for NTDs (Detrait et al., 2005). This led to the discovery of an inverse correlation between NTD risk and maternal serum levels of folic acid during pregnancy, with a threshold of 200 µg/l, below which the risk for NTDs

becomes very significant. A 400 to 800 µg/day dietary supplementation of folic acid for women who plan to become pregnant has shown a reduction in the incidence of NTDs of more than 75% (Nussbaum et al., 2001).

In general, NTDs present a high clinical and etiologic heterogeneity. Their classifica-tion according to the multisite closure model to a large extent allows one to explain this diversity in defects, by suggesting the possi-bility of a distinct genetic control for each clo-sure point, as well as different sensitivities of these points to environmental factors. Thus, piece number 496 from the collection of the Federal University of São Paulo – Paulist Medical School – presents a closure defect at site 2 (Van Allen et al., 1993), reaching the parietal region of the head, prosencephalon and mesencephalon. The symmetrical and per-fect architecture of the borders allows it to be confirmed that there was indeed a primary malformation in neural tube closure. The presence of an anterior fontanelle with an aver-age diameter greater than the second positive deviation for the estimated age range, the par-allel asymmetry between the coronal sutures, and the deviation of the median line of the frontal suture could be consequences of pri-mary malformation in neural tube closure. There are many etiologies associated with NTDs involving site 2, among them maternal use of carbamazepine, aminopterin, alcohol abuse, type I diabetes mellitus, among others (Table 1). Currently, regarding deficiency of folate nutritional factors, its daily supplemen-tation in the mother's periconceptional diet does not reduce the risk for NTDs by 100% but it does minimize the risk significantly. This shows that the etiology of NTDs is com-plex and involves not only a single gene, but the interaction of multiple and specific genet-ic and environmental factors.

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