

## ORIGINAL PAPER

# Macro- and microanatomic factors involved in the flat bones morphogenesis from the craniofacial system

MIHAELA IUSTINA MEȘINĂ-BOTORAN<sup>1)</sup>, C. MEȘINĂ<sup>2)</sup>, G. S. DRĂGOI<sup>1)</sup>,  
L. MOGOANTĂ<sup>3)</sup>, R. L. NEAMȚU<sup>1)</sup>

<sup>1)</sup>Department of Human Anatomy

<sup>2)</sup>Department of Surgical Semiology

<sup>3)</sup>Department of Histology

University of Medicine and Pharmacy of Craiova

### Abstract

The authors present a study both upon eight crania of dead born fetuses having the vertex–cocci distance between 25–29 cm and a human embryocephalic extremity of 18 cm. The crania were examined in the lateral, vertical, frontal, occipital, basal norms. Sagittal sections were performed upon the embryo, and then those sections were microscopically examined after HE staining. We concluded that the cranial arch bones morphogenesis and the facial complex is a long-time development process, which initially started during the early embryogenesis, and it is completed as an adult. Determining factors of the flat bones osteogenesis are the following: vascular, muscle, extracell (mesenchyma) and neuronal factors (rhombencephalon presence).

**Keywords:** bones morphogenesis, flat bones, osteogenesis.

### Introduction

Membranous neurocranium corresponds to the cranium arch and it is not performed in cartilage. Its elements, frequently described as dermal bones, are the frontal, parietal bones, the squamous part of the temporal bones and the upper part (interparietal) of the occipital squama. The frontal, occipital and the squamous bones are thought to be made up of the neural crests [1, 2].

There is a close association between the meninges development, particularly dura mater and calvaria bones.

Dermal bones are made by initiating the osteodifferentiation wave radially moved from the ossification centres into the desmocranial mesenchyma inner [3]. When the adjacent bones meet, the osteogenic front proliferation stops and the suture formation is induced. Once the sutures are formed and the desmocranium is replaced by mineralized bone, the second development phase appeared where the cranial bones growth develops at the level of the sutural borders [4]. Through such a growth the most part of calvaria is formed.

### Material and methods

The flat bones of the cranium were removed from eight dead born fetuses between 1995–2001 at the „Filantropia” Hospital of Craiova; vertex–cocci distance were between 25–32 cm. Cephalic extremity of a 18 mm human embryo was microscopically examined on sagittal sections.

The images were then processed by Lucia M specialized licensed soft, in the Laboratory of Imagistic and Analysis of the Department of Human Anatomy within the Faculty of General Medicine of Craiova. That soft allowed us morphometric determinations and their statistic processing by its specific functions.

### Results

A main part in determining the flat bones stereotopography is played by the periosteum and sutures within the craniofacial system morphogenesis.

From macroscopic analysis of the cephalic extremities removed from human fetuses, we noted many anatomic-descriptive, anatomic-topographic and structural characteristics such as:

- by examining a fetus cranium with vertex–cocci distance of 25 cm and 27 cm respectively (Figures 1 and 2) under the lateral norm we easily noted the external face of the squamous process of the occipital bone, which is convex posteriorly and articulates to the occipital border of the both parietal bones at the level of the lambdoide suture. Sagittal border of the parietal bone articulates the opposite parietal bone at the level of sagittal suture. At the level of the occipital bone squama, parietal bone and also at the level of temporal squama, the periosteum is poorly adherent but it is strongly adheres the fibrous tissue of the sutures among them (occipito–parietal, interparietal);

- by examining a 25 cm vertex–cocci distance fetus cranium under the occipital norm (Figure 2) we revealed the occipital parts of both the parietal bones, which are

dentate, joining the occipital bone squama at the level of the lambdoid suture and the sagittal border of each parietal bone joins that of the opposite parietal bone, at the level of the sagittal suture. Periosteum from the level of parietals was easily removed. On that piece we noted cranial dura mater strongly adherent at the level of both lambdoidal and sagittal sutures, but easily enough detachable;

- by examining a 27 cm vertex–coccyx distance fetus cranium under the frontal norm, we easily noted the metopic suture, at the lower part of the middle line; by examining a 25 cm vertex–coccyx distance fetus cranium (Figure 3) under the frontal norm, we noted the periosteum which is very adherent at the level of the metopic suture;

- by examining a 29 cm vertex–coccyx fetus cranium (Figure 4) under the basal norm, we easily noted the periosteum adherent in the anterior level of the exobase;

- by examining a 29 cm vertex–coccyx fetus cranium (Figure 5) under the vertical norm we established the oval shape the most sizable part of the oval being in the occipital region. Periosteum was easily detached from the parietal bones being adherent at the level of the sagittal, lambdoidal and coronal sutures. We noted dura mater presence on the endocranial face of the frontal bone squama.

Stereotopographic analysis and structural particularities of the neurocranium (cephalic extremity – a 18 mm embryo) was studied by microscopic examination of the cephalic extremity of a human embryo (Figure 6) with ob.  $\times 10$  of the Hematoxylin–Eosin staining section; we noted the relationships between pia mater and the choroidal plexus elements in the rhombencephalic region. In the lower third of the image, the occipital is partially formed by enchondral ossification process.

## ☞ Discussions

Intramembranous bony growth is achieved by bony formation inside the periosteum or at the level of sutures. The latter are formed during the embryonic development as proximity places of the membranous bones of cranio-facial skeleton. They are used as major places of bony expansion during the postnatal craniofacial growth.

By using the chimeric culture growth system of quail chickens, Couly GF *et al.* demonstrated the origin of the membranous bones of cranium (frontal, parietal and the squamous parts), of their sutures of the suprajacent derma and subjacent dura mater as being from the early migrated population of the neural crest cells [5].

By using the same system of quail chickens chimeric culture and more recently by using viral transfection Nodden showed that intramembranous bones of the cranium arch, their suprajacent periosteum and neighboring sutures would be of paraxial mesoderm origin while the subjacent duramater derived from neural crest [4, 6, 7].

Susceptibility opening to induction is relatively short. Once the induction occurred, the continuous

presence of the inductive tissue is not necessary for a long time [8, 9]. Unless the neural crest cells directly contribute to intramembranous bone formations of the cranium arch, they would indirectly contribute by means of dura mater, which undoubtedly was born from the neural crest cells [9].

It was demonstrated that neonatal dura mater can determine a bony formation then it was transplanted under the epithelium but not when it was transplanted more profoundly under the mesoderm derived from the tissular layers [10].

Some authors, put forward the hypothesis that dura mater can induce bony formation in the epithelium presence and is very likely that the neonatal epithelium to induce bony formation by dura mater [11].

The fact is very interesting that these experiments seem to emphasize a longer inductive period than that described before. After all probabilities, the major consequence of those inductive influence is the presence of a tissue sufficiently enough to respond those inductive influences.

Once the cranium bones condensations appeared and the ossification went them on, the next important event would occur when the bones were closed to each other and the suture formation would be initiated [12, 13].

In this time, the sutures would become the major places of the intramembranous bony growth, neonatal and postnatal during the rapid expansion of the neurocranium and the maxillary complex development.

After the induction of the osteogenic potential, intramembranous bony formation initiation went on by the development of the mesenchymal blastema representing the forerunner of the each cranium arch bone. However, as the ossification went on and the neural growth was reduced, the bony fronts got closed to each other and thus the suture formations was initiated as a bony front neighboring to each other, with the fontanelles representing unossified regions of the confluence of more than two bones of the cranial arch.

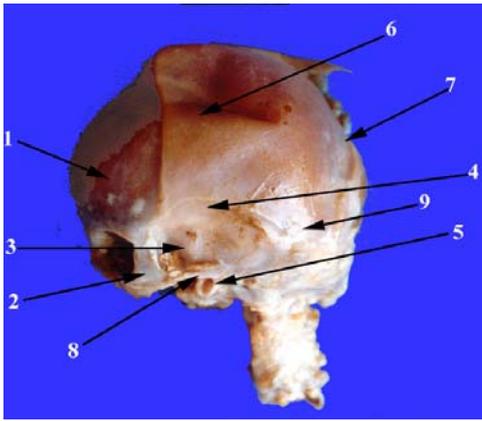
During the development of sutures, the growth and extension of bony fronts invades but also completes the neighboring mesenchymal tissue to advanced borders of bony fronts. Though dura mater is not necessary to the initial overlapping of the bony fronts during the coronal sutures development, its presence is necessary for initial stabilization of the suture [10].

Medial sutures (sagittal, interfrontal) are the so-called „hinge-sutures” which do not overlap, while the sutures transversally situated (lambdoidal, coronal) are overlapping sutures.

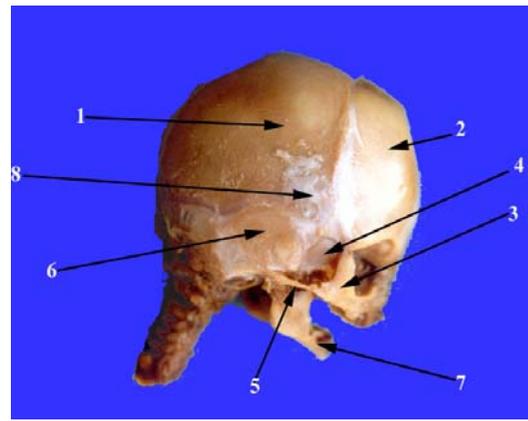
Now it is thought the bony front getting close is made based on the growth factor gradient signalized between them, which initiate the suture formation [13].

Those results indicated that dura mater allows sutural formation but an inductive stimulus from dura mater is necessary during the sutural formation, before the suture could maintain by itself [14].

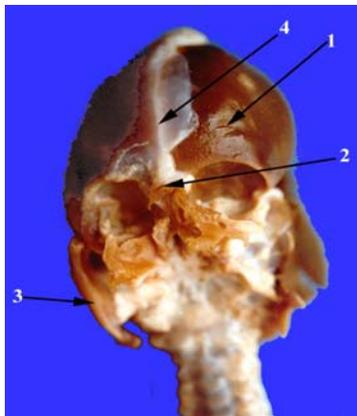
One of these possibilities is that dura mater is strictly derived from the neural crest and the periosteum has some contributions from the paraxial mesoderm.



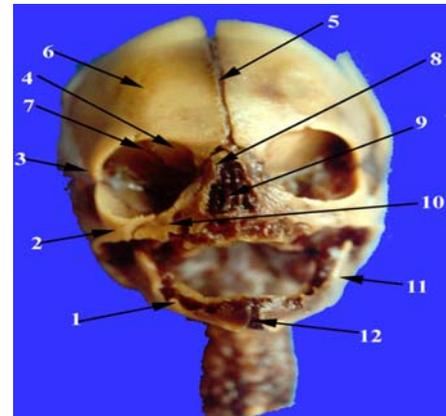
**Figure 1** – A fetus cranium with vertex–coccyx distance of 27 cm in lateralis norma: 1 – squamous process of the frontal bone; 2 – zygomatic bone; 3 – ala major of the sphenoidal bone; 4 – squamous process of the temporal bone; 5 – anulus tympanicus; 6 – parietal bone; 7 – squamous process of the occipital bone; 8 – zygomatic arcade; 9 – periosteum



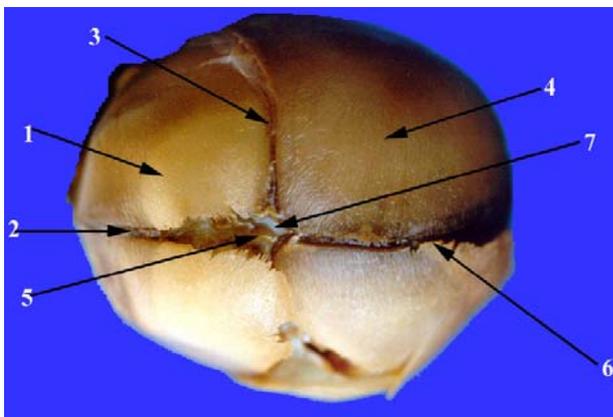
**Figure 2** – A fetus cranium (V – C = 25 cm), norma lateralis: 1 – parietal bone; 2 – squamous process of the frontal bone; 3 – zygomatic bone; 4 – ala major of the sphenoidal bone; 5 – zygomatic arcade; 6 – squamous process of the temporal bone; 7 – left hemymandibula; 8 – periosteum



**Figure 3** – A fetus cranium (V – C = 25 cm), frontalis norma: 1 – squamous process of the frontal bone; 2 – nasal process of the frontal bone; 3 – left hemymandibula; 4 – periosteum



**Figure 4** – A fetus cranium (V – C = 27 cm), in frontalis norma: 1 – right hemymandibula; 2 – right zygomatic bone; 3 – squamous process of the frontal bone; 4 – orbital process of the frontal bone; 5 – metopic suture; 6 – squamous process of the frontal bone; 7 – superior orbital fissure; 8 – nasal bone; 9 – inferior nasal cornet; 10 – maxillary bone; 11 – left hemymandibula; 12 – mentonier symphisial crest



**Figure 5** – A fetus cranium (V – C = 29 cm), verticalis norma: 1 – squamous process of the frontal bone; 2 – metopic suture; 3 – coronar suture; 4 – parietal bone; 5 – fonticulus anterior; 6 – fonticulus posterior; 7 – dura mater



**Figure 6** – Stereotopographic analysis and structural particularities of the neurocranium: the relationships between pia mater and the choroidal plexus elements in the rhombencephalic region. In the lower third of the image the occipital is partially formed by enchondral ossification process

Another possibility is that all the tissues of the subepidermic cranium originate into the neural crest and ectoperiosteum becomes associated to the bony and dermic formation while dura mater becomes associated to bony and brain formation. We also noted the fact that facial sutures, which appear very similar to those of the cranium arch, concerning both the function and morphology, they do not contact a subjacent dura mater [15].

As the brain expands and the sutures of the cranium base elongate, the sutures respond by adding intramembranous bone at the borders of the bony fronts so that the sutures are of the same size, the cranium arch increases thus becoming put up with the brain expansion. For the sutures behave as bony growth places they have to get an unossified status, thus allowing new bone formation at the level of the neighboring bony fronts borders. This process achieves a new bony cell production enough to be recruited into the bony fronts while the cells rested into the suture remain undifferentiated [8].

### ☐ Conclusions

It is possible that the pericondrium and cartilages regulate the bony formation at the level of the facial sutures in a similar way to brain and dura mater at the level of the cranium arch sutures.

Angiogenesis is an essential component of the flat bones development and plays a main part, if not a central one in this process.

Intramembranous bony growth is achieved by bony formation inside the periostum or by bony formation at the level of sutures.

In the case of the facial sutures the bones growth one toward the other by a lax mesenchymal tissue, while in the case of the cranium arch sutures, the bones get closed to each other by means of a preformed fibrous membrane.

Cranium sutures, during the process of bony growth respond by adding intramembranous bone at the level of the bony front borders, so that the cranium arch develops according to the brain expansion.

For the sutures behave as bony growth places, they have to get an unossified status, thus allowing the new bone formation at the level of the neighboring bony fronts borders.

In the absence of the “growth cartilage” from the long bone structure, the flat bone changes of calvaria achieves by osteogenerative activity of the fontanelles, which go on during the postpartum stage of ontogenesis.

### Corresponding author

Mihaela Iustina Meşină-Botoran, Assistant Professor, MD, PhD, Department of Anatomy, University of Medicine and Pharmacy of Craiova, 2–4 Petru Rareş Street, 200349 Craiova, Romania; Phone +40251–524 442, E-mail: mesina.cristian@doctor.com

Received: January 16<sup>th</sup>, 2007

Accepted: September 15<sup>th</sup>, 2007

### References

- [1] DRĂGOI G. S., MOCANU G., FERSCHIN A., MEŞINĂ C., STĂNESCU M. R., “Coincidence of contraries” in balancing the processes of genesis and extracellular matrix evolution in the bony structures, anniversary volume, University of Medicine and Pharmacy of Craiova, May 24–25, 2002, Medical Publishing House, 38.
- [2] DRĂGOI G. S., MEŞINĂ C., MOCANU G., *Anencephalon between theory and reality*, “Victor Babeş” Morphology Symposium, November 5–7, 2002, 506.
- [3] DRĂGOI G. S., MEŞINĂ C., MIHAELA IUSTINA MEŞINĂ, PĂRVĂNESCU H., *Theoretic problems about ectopic ossification*, “Victor Babeş” Morphology Symposium, November 5–7, 2002, 32.
- [4] OPPERMAN L. A., CHHABRA A., NOLEN A. A., BAO Y., OGLE R. C., *Dura mater maintains rat cranial sutures in vitro by regulating suture cell proliferation and collagen production*, J Craniofac Genet Dev Biol, 1998, 18(3):150–158.
- [5] COULY G. F., COLTEY P. M., LE DOUARIN N. M., *The triple origin of skull in higher vertebrates: a study in quail–chick chimeras*, Development, 1993, 117(2):409–429.
- [6] NODEN D. M., *Cell movements and control of patterned tissue assembly during craniofacial development*, J Craniofac Genet Dev Biol, 1991, 11(4):192–213.
- [7] OPPERMAN L. A., PASSARELLI R. W., NOLEN A. A., GAMPPER T. J., LIN K. Y. K., OGLE R. C., *Dura mater secretes soluble heparin-binding factors required for cranial suture morphogenesis*, In Vitro Cell Dev Biol Anim, 1996, 32(10):627–632.
- [8] RICE D. P., KIM H. J., THESLEFF I., *Apoptosis in murine calvarial bone and suture development*, Eur J Oral Sci, 1999, 107(4):265–275.
- [9] YU J. C., MCCLINTOCK J. S., GANNON F., GAO X. X., MOBASSER J. P., SHARAWY M., *Regional differences of dura osteoinduction: squamous dura induces osteogenesis, sutural dura induces chondrogenesis and osteogenesis*, Plast Reconstr Surg, 1997, 100(1):23–31.
- [10] ROTH D. A., BRADLEY J. P., LEVINE J. P., McMULLEN H. F., MCCARTHY J. G., LONGAKER M. T., *Studies in cranial suture biology: part II. Role of the dura in cranial suture fusion*, Plast Reconstr Surg, 1996, 97(4):693–699.
- [11] BRADLEY J. P., LEVINE J. P., BLEWETT C., KRUMMEL T., MCCARTHY J. G., LONGAKER M. T., *Studies in cranial suture biology: in vitro cranial suture fusion*, Cleft Palate Craniofac J, 1996, 33(2):150–156.
- [12] HALL B. K., MIYAKE T., *All for one and one for all: condensations and the initiation of skeletal development*, Bioessays, 2000, 22(2):138–147.
- [13] ISEKI S., WILKIE A. O., MORRIS-KAY G. M., *Fgfr1 and Fgfr2 have distinct differentiation- and proliferation-related roles in the developing mouse skull vault*, Development, 1999, 126(24):5611–5620.
- [14] LEVINE J. P., BRADLEY J. P., ROTH D. A., MCCARTHY J. G., LONGAKER M. T., *Studies in cranial suture biology: regional dura mater determines overlying suture biology*, Plast Reconstr Surg, 1998, 101(6):1441–1447.
- [15] SPERBER G. H., *Pathogenesis and morphogenesis of craniofacial developmental anomalies*, Ann Acad Med Singapore, 1999, 28(5):708–713.