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Prenatal craniofacial morphogenesis: four-dimensional visualization of morphogenetic processes

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Structured Abstract

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Objectives – Basic research concerning craniofacial development presently runs along two pathways, namely the molecular and the morphometric. This gap needs to be bridged.

Design – Using histological serial sections of human fetuses computer-aided three-dimensional reconstructions were made (Software Analysis, SIS) with special focus given to all anatomical structures of the orofacial region of the growing head.

Results – All reconstructions can be viewed from any rotation and they are available for virtual dissection according to anatomical rules. As an example, the prenatal development of the human mandible with the formation of the mental foramen therein is described. Furthermore, the spatial arrangement of bone, cartilage and nerves is presented in three dimensions in different developmental stages. The interaction of tissues with possible morphogenetic interaction is discussed.

Conclusions – This work serves as a reference system for prenatal development in comparison with pathological development.

Key words: craniofacial morphogenesis; human; prenatal

To cite this article:
Orthod Craniofacial Res **6** (Suppl. 1), 2003; 89–94
Radlanski RJ:
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ISSN 1741-2420

Introduction

> ‘*The determination of shape and size is one of the biggest unsolved problems in developmental biology*’
> [Day and Lawrence 2000 (1)].

Until recently, there has been little information regarding the mechanisms that govern the assembly of the facial primordia. The very small size of early embryos precludes their direct analysis, leaving few options for investigation. One approach is to use three-dimensional (3-D) reconstruction of human embryos from histological serial sections. Embryos and fetuses ranging in size from 18 mm (measured as crown-rump length, CRL) to 290 mm CRL have been studied in the laboratory. For the majority of the 3-D reconstructions the software analySIS by Soft Imaging Systems, Münster, Germany has been utilized. By means of the approach of virtual dissection of human embryos it is possible to gain insights into the mechanisms of complex craniofacial architecture that are not obvious from two-dimensional (2-D) analyses.

Craniofacial morphogenesis is a process that takes place in space and time. Therefore, the description of the observable changes should be given in 3-D consequently (2). In this way it is possible to describe the changes at the macroscopical morphologic and microscopic cellular levels. In cooperation with other research groups laboratory hopes to localize the modality and sources of the signals, their timing, and their distances to be covered between origin and target. In many regions of the developing human face the quality of the morphological description of the developmental processes is lagging behind the general progress that has been made in the field of molecular biological research. It is the aim of work in laboratory to gain detailed morphological information of the craniofacial developmental process regarding all tissues (nerves, vessels, bone, mesenchymal tissue). The goal is to draw together molecular biology and morphology.

The 3-D reconstruction technique

The technique of 3-D reconstructions from histological serial sections (Fig. 1a) was introduced by Born (3), who built wax plate models. This technique was modified by Blechschmidt (4) who built up a large collection of 3-D reconstructed human embryos in glass showcases in Göttingen, Germany. Modern computer technology enables us to make use of computer-aided 3-D reconstructions (5–7). The contours of the selected anatomic structures are traced under the microscope and entered into the computer (Figs 1b, c). With this technique the specimens can be viewed from any angle on the computer screen, and electronic dissection enables us to omit single sections or to select anatomical structures to make them transparent (Figs 2a, b) or to take them away (Fig. 2c).

The value of the 3-D technique

Human embryological research made a quantum leap when the first scientific 3-D reconstructions were made (3, 8–10). The speculations of Haeckel's Biogenetic law (11), after which ontogeny was believed to be a recapitulation of phylogeny, could then be replaced by scientific findings accurately describing human (and animal) embryos at different developmental stages and

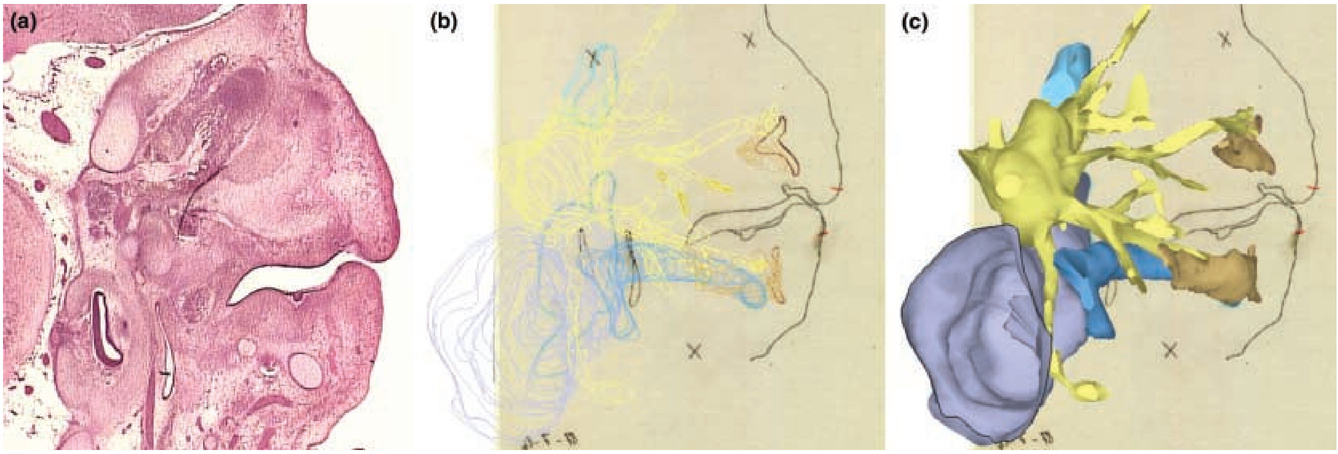

Fig. 1. (a) Sagittal histological section (out of a series of sections) of the facial region of a human embryo of 20 mm crown-rump length. (b) The contours of each single section are traced under the microscope and entered into a computer (software: analysis by SIS). Three-dimensional reconstruction in line mode. (c) Rendered 3-D reconstruction; trigeminal ganglion and trigeminal nerve, temporal bone (pars petrosa, lower left), Meckel's cartilage and mandibular bone, maxilla, contour of the face (right).

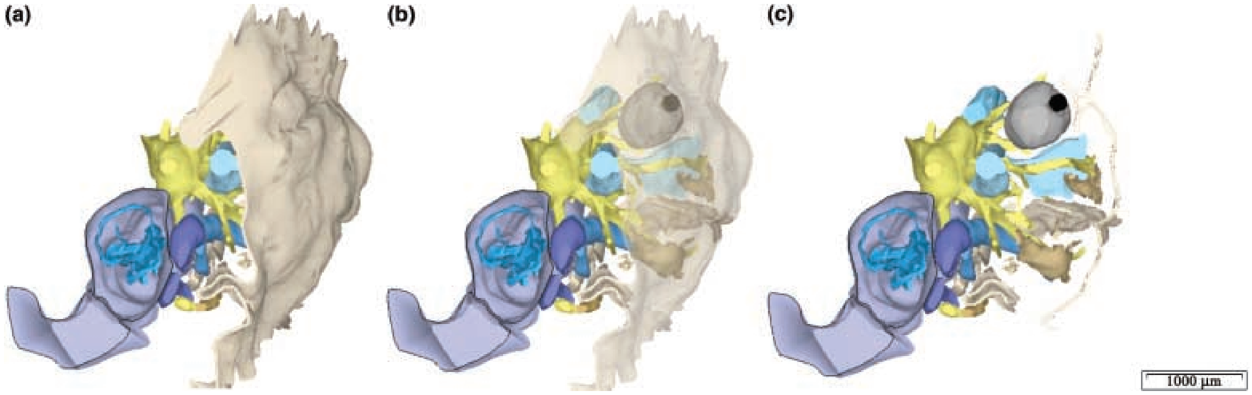

Fig. 2. (a) Three-dimensional reconstruction of the facial region of a human embryo, 20 mm crown-rump length. The facial skin covers the skeletal components. (b) The same reconstruction with transparent skin. (c) The same reconstruction with the skin removed in order to view the skeletal facial components, the trigeminal nerve, the eye, and the oral cavity (bar: 1 mm).

from inside. It was not until the 1960s that Haeckel's 'law' was refuted, and for the scientific public to be told that human fetuses at no time have gill slits, not even as ancestral remnants (12–14).

Human craniofacial morphogenesis is a very complicated process in space and time, and computer-animated sequences (7, 15, 16) have proven to be an excellent teaching tool for students.

For basic research in the field of craniofacial morphogenesis, detailed reconstructions in 3-D, covering the same structures over different developmental stages, reveal changing proportions of the components, neighborhood relationships and possible mutual influences.

Until now, the widely expanding insights in the field of molecular biology of craniofacial research, are mostly presented in 2-D sections or in the form of schematic drawings. It would be much more sensible to transfer these findings into a 3-D arrangement. This way, real extensions of tissues and their signaling properties can be viewed, real distances between putatively interacting tissues can be evaluated, and the gap between molecular biology and *morpho*genesis is expected to be bridged.

Development of the mandible

It is well known that Meckel's cartilage forms in the center of the first visceral arch (17) and serves as a framework for the mandibular bone forming lateral to it. On the molecular level, certain patterning of the early face could be revealed (18). We know that FGF-mediated signaling plays a fundamental role in the proliferation and skeletogenic differentiation in very early stages of facial development. And, next to an abundance of other functions, bone morphogenetic proteins play a role in bone formation. However, how is the form of the early bony mandible controlled (Fig. 3)? What leads to the broadening of Meckel's cartilage from the acute v-shape to the wide u-shape (Figs 3 and 4)? (19). The early bone remodeling patterns during prenatal mandibular development have been described recently (20). Mandibular bone follows almost exactly the gently undulated path of Meckel's cartilage while expanding into anterior and posterior directions. This is only visible in morphologically correct 3-D reconstructions and they reveal the certainty that spatial conditions may play a role in morphogenesis (8, 21, 22).

There is a theory that characterizes the mesenchymal tissue whose fate it is to become bone as being located in such an area that is typified by distraction forces exercised by neighboring tissues (4, 21). For the

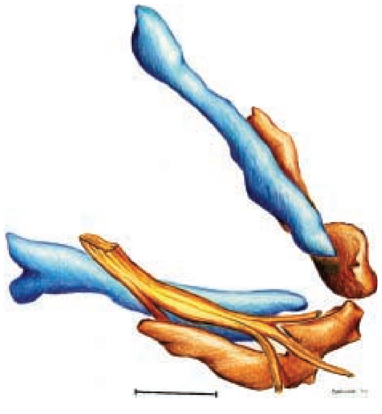

Fig. 3. Three-dimensional reconstruction of the mandible with Meckel's cartilage and alveolar inferior nerve of a human embryo of 22 mm crown-rump length (bar: 250 mm).

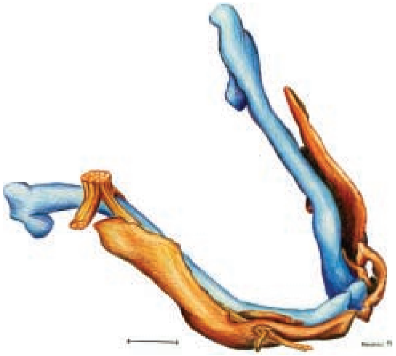

Fig. 4. Three-dimensional reconstruction of the mandible with Meckel's cartilage and alveolar inferior nerve of a human embryo of 25 mm crown-rump length (bar: 250 mm).

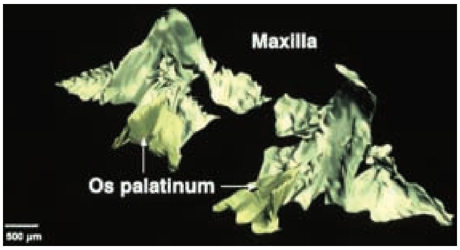

Fig. 5. Three-dimensional reconstruction of the maxilla of a human embryo of 25 mm crown-rump length; posterior, 45° cranial and 45° lateral view (bar: 500 μm).

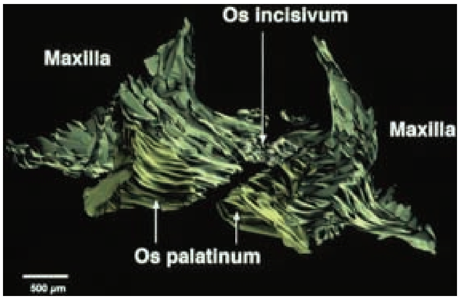

Fig. 6. Three-dimensional reconstruction of the maxilla of a human embryo of 68 mm crown-rump length; posterior, 45° cranial and 45° lateral view (bar: 500 μm).

mandible, this is easy to understand, because the longitudinal expansion of Meckel's cartilage against the adjacent skin tissue leaves a narrow layer of mesenchymal tissue which underlies a vector of shearing force (23). This might act as a signal for the cells to react in the way recent molecular studies revealed.

The mental foramen first looks like a u-shaped notch and later it is encircled by bone (Figs 3 and 4). No information is available to explain what triggers bony tissue to cover the inferior alveolar nerve in that pattern. For further research, the formation of a structure, which is not too complex but multifaceted enough, like mental foramen, can serve as a model to study the activity of signaling agents during morphogenetic processes. As a first step, the morphological details and relationship between the neighboring tissues have been described (24). Detailed descriptions of the remodeling processes (20) with special focus on the foramen region should follow, and it can be well expected that signaling patterns will be found on the cellular and the molecular level. Finally, it is hoped to be able to localize and to correlate the molecular biological processes in 3-D with the morphological changes in.

Development of the maxilla

The outline of the bony tissue in the maxilla (Figs 5 and 6) (25) almost completely resembles the spatial conditions that remain after the epithelial structures have molded the palatal roof of the oral cavity, the floor of the nasal cavity, and to either side of that cavity, the space which is occupied by the eyes. According to the above described theory, that bone forms in regions that underlie shearing force vectors (4, 21), the 3-D reconstructions show the expanding oral cavity, the widening nasal cavity and the enlarging eyes with bone formation exactly in the spaces left between. Further research, with correlation of molecular signaling and allocation in 3-D are expected to shed light on this putative interdependency.

Developmental movements, patterning, and morphogenesis

A series of 3-D reconstructions of fetal facial development with the major hard and soft tissues included show the proportional changes that occur from 31 mm CRL (Figs 7a, b), over 53 mm CRL (Fig. 8a) to 117 mm CRL (Fig. 9a, b). It is possible to observe the changing outlines – the shapes – the *gestalt* of the involved tissues and organs during their development through different stages, and performance of developmental movements (4, 26). There is a chance to evaluate how much organ forming processes are controlled independently from surrounding structures, and to what extent there is a competition for extending into the available space, leading to formation of a specific shape. For the development of the major salivary glands it is plausible that expansion is into spaces that

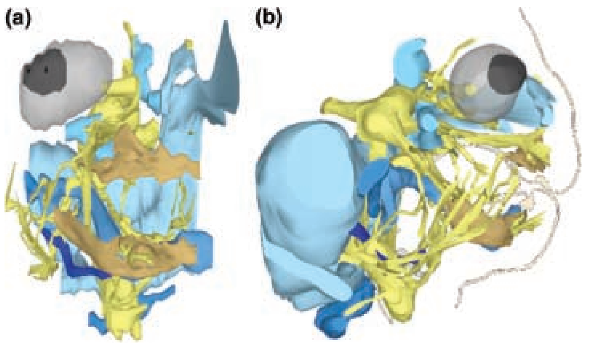

Fig. 7. (a) Three-dimensional reconstruction of the facial region (skin removed) of a human embryo of 31 mm crown-rump length; frontal view, right half of the face; diameter of the eye: 1 mm. (b) Right lateral view.

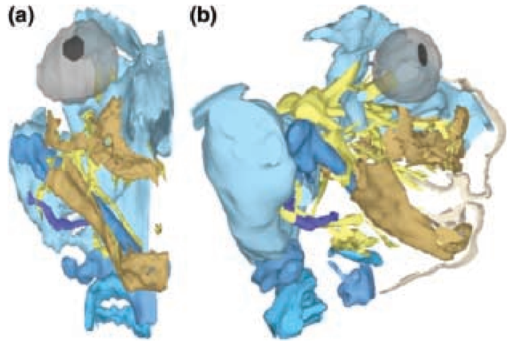

Fig. 8. (a) Three-dimensional reconstruction of the facial region (skin removed) of a human embryo of 53 mm crown-rump length; frontal view, right half of the face; diameter of the eye: 1.5 mm. (b) Right lateral view.

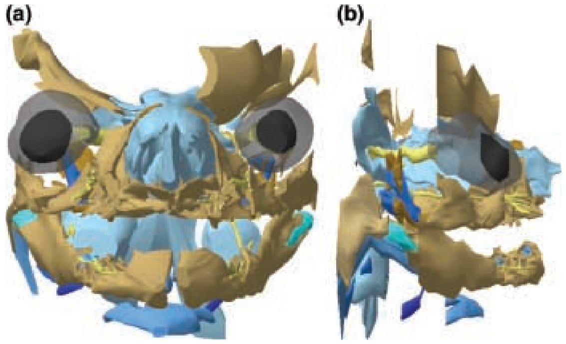

Fig. 9. (a) Three-dimensional reconstruction of the facial region (skin removed) of a human embryo of 117 mm crown-rump length; frontal view, diameter of the eye: 2 mm. (b) Right lateral view.

are limited by neighboring structures (27, 28). Another area to consider regarding the arrangement of developing structures of the muscles of the floor of the mouth: here, it might be that the orientation of fibers are in alignment to the vectors of expansion of the mandibular frame in transversal, sagittal, and vertical directions (29).

Research on early facial development deals with patterning (18, 30–32). Although an abundance of genes involved has been revealed, and a complex of interacting signaling proteins has been identified, the situation is too complicated to expect a simple explanation for facial morphogenesis. It is without doubt that there is a species-specific regulation pattern that forms a typical human face. In monozygotic twins faces closely resemble each other, but parents as well as acquaintances can still identify the individual twins easily, because their faces do show individual morphological traits. Here the epigenetic influence becomes obvious, but in detail it is not at all clear how this is generated, while the signaling patterns are believed to be genetically controlled (33–37).

It has been explained that a very well distinct activity of sonic hedgehog (SHH) and retinoic acid is required to form a normal face (33, 38). A deficiency of SHH at a certain developmental stage leads to hypotelorism and clefting, a surplus leads to mediolateral widening and duplications. As clear as this interdependency seems to be, a correlation to what happens to the tissues involved and a visualization of their location and activity projected on an exact 3-D reconstruction would help in the understanding of the causal chain of events on both levels, molecular and morphological! (39, 40).

Clinical utility and implications

Three-dimensional reconstructions of craniofacial development are very helpful in teaching the complex morphological peculiarities and developmental changes to students. Moreover, the role of molecular influences in normal and abnormal (41) developmental processes will be clarified on the morphological level. To that end, species-specific human development must be compared with the developmental processes of experimental animal models, predominantly mouse and chick. In the future, prenatal diagnosis and possible surgical interventions are expected be possible.

Acknowledgements: I am indebted to Dr H. Renz for most valuable contributions.

I thank Mrs B. Danielowski and Mrs I. Schwarz for their competent technical assistance. This work was supported by grants from Quintessence, Dentaurum, and, in parts, DFG (Deutsche Forschungsgemeinschaft) Ra 428/1-3, furthermore by COST B8.

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