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3D-µCT Cephalometric Measurements in Mice

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1. Introduction

The skull of all vertebrates is a structure made up of the neurocranium, which surrounds and protects the encephalon, and the viscerocranium, which protects the initial segment of the digestive and respiratory systems. The separate bones that form the skull are articulated among them forming sutures and synchondroses in the adjacent margins of the membrane bones of the calvaria and of the bones of the skull base, respectively (see for a detailed review and references Wilkie and Morriss-Kay, 2001; Morriss-Kay and Wilkie, 2005).

Advances in molecular genetics over the past two decades have revealed some of the key genes for skull vault development (Verdyck et al., 2006). Then, the genetic engineering has been used to construct mice that lack these genes resulting in abnormal craniofacial development, equivalent to those of some human conditions. Therefore, the murine model has been chosen as a surrogate for studying the biologic behavior of human cranial bones and joints-sutures. For example, we have recently analyzed the cranial, mandible and tooth defects of a mouse strain which mimics a human progeroid syndrome (De Carlos et al., 2008). These mouse models are basics for understanding the developmental mechanisms leading to skull malformations, and may eventually help in the development of new therapeutic strategies.

The image technique modalities used to quantitatively asses the changes in size and shape in the skull in these animal varies from simple radiology to three-dimensional (3D) micro-computed tomography (µCT; Figure 1; see for a review Tobita et al., 2010). Nevertheless, 3D-µCT is becoming more and more a common technique for the anatomical analyses of these mice models (Paulus et al., 2001; Song et al., 2001; Recinos et al., 2004; Schambach et al., 2010), especially in the field of the skeletal development and growth (Guldberg et al., 2004). For example, 3D µCT quantitative evaluations have been made in mouse to study different functional skull changes (Enomoto et al., 2010; Saito et al., 2011a,b), or several kinds of developmental or genetic skull malformations (Perlyn et al., 2006; De Carlos et al., 2008;

Coleman et al., 2010; Purushothaman et al., 2011), or the distribution of some genetic characters in different strains of mice (Nishimura et al., 2003).

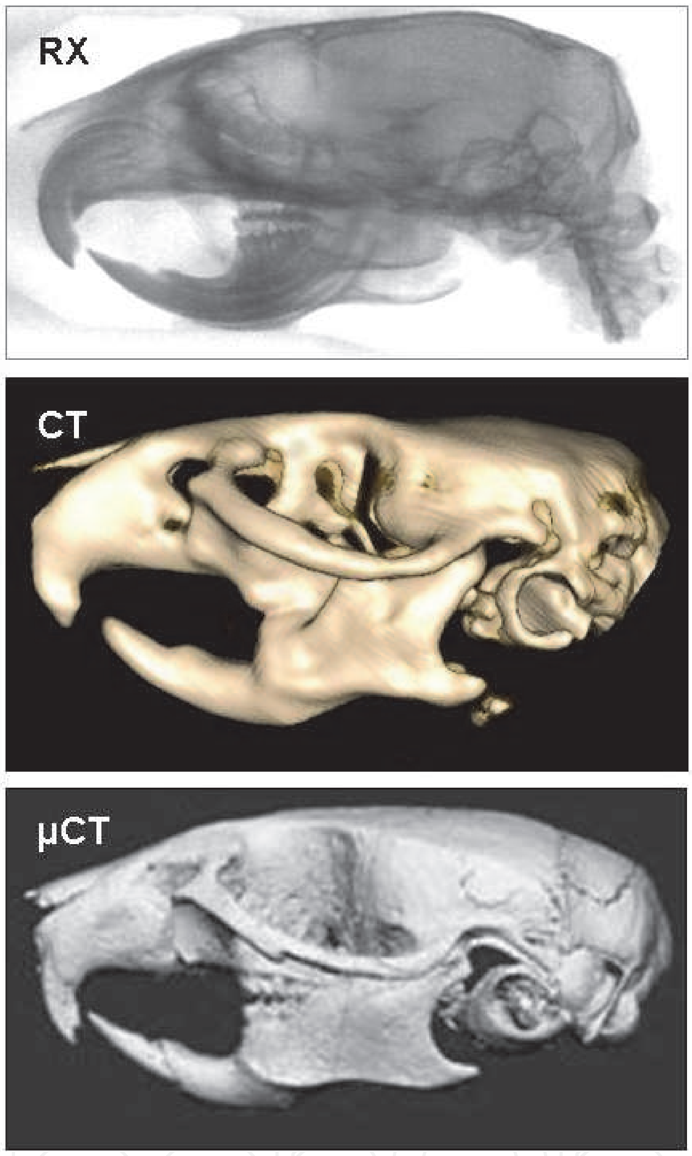

Fig. 1. Lateral view, left, of a mice skull using simple radiology (RX), conventional computed tomography (CT), and micro-computed tomography (μCT). Only simple radiology and μCT show detailed morphology of the skull and therefore consent an accurate localization of landmark points for cephalometry.

Cephalometric radiography analyses have been developed for the evaluation of specific skull of rodents, but no comprehensive standardized cephalometric methods have been generated for mice. Moreover, most of the 3D-μCT studies were used to show differences between wild-type and mutated mice evaluating a few number of lineal or volumetric parameters. These measurements are sufficient to quantitatively evidence the main skull changes induced by an experimental manipulation but are insufficient to accurately evaluate the length, height and width of the different segments of the cranium and the mandible. Thus, we consider that the skull measurements in mice must be more detailed in order to acquiesce to all the skull defects induced by an experimental condition or a mutation.

In this chapter we first establish landmarks which can be easily identified in 3D-μCT images from mice skull. Thereafter, in order to define the skull phenotype we propose a cephalometric study based on the osseous landmarks currently used in human orthodontics and orthopedics. Also we compare the results of cephalometric measurements obtained using simple radiology and those obtained using 3D-μCT. Finally, we underline the advantages and disadvantages of 3D-μCT for evaluating the morphology of mice skull. The 3D-μCT database of the skull size and shape in different mouse strains are necessary to provide references for future studies involving large-scare mutant screening.

2. Localization of cephalometric landmarks in mice skull using μCT

To perform 3D-μCT cephalometric analysis the first step is the identification and localization of cranial and mandible reference landmarks directly on the bone surfaces. Accurate location of landmarks and user skill are important factors to achieve reliable data. Here we have identified a series of landmarks than can be extrapolated to those used in human cephalometric, and therefore consent a detailed measurement of the mice skull. Some authors (Nishimura et al., 2003), however, limit cephalometric analysis to a small number of reliable and informative landmarks.

To perform cephalometric study we purpose, the the following landmarks were identified (Figure 2):

Norma dorsalis o superior (Fig. 2A): 1: internasal point; 2: occipital point; 3: nasal points; 4: orbital point (right and left infraorbital foramina); 5: zygomatic points; 6: jugal process of squamosal bone.

Norma basalis (Fig. 2B)**:** 7: interdental point; 8: posterior nasal spine.

Norma posterior (Fig. 2C): 2: occipital point; 5: zygomatic points; 6: jugal process of squamosal bone.

Norma anterior (Fig. 2D)**:** 4: orbital point (right and left infraorbital foramina); 5: zygomatic points.

Norma lateralis (dextra; Fig. 2E): 9: naso-maxillary point; 10: superior incisor-alveolar point; 11: prostion; 12: superior incisor point; 13: parietal point; 14: tympanic point.

The use of 3D-μCT imaging allows for the demonstration of structures and landmarks that are impossible to identify by conventional radiographic methods. It also allows for the selection of images at any desired angulation, and the calculation of 3D distance between any two points. Of particular interest are measurements that cannot be easily obtained by plain radiographs, such transverse distances between the same points on the two sides of the maxilla or mandible.

3. A proposal for the cephalometric analysis by μCT in mice

The dimensional analysis of the skull using 3D-μCT is based on measurements between reference landmarks, whereas topological analyses provide 3D geometrical reference frames using the reference landmarks. The shape measurements can be defined by ratios of inter-landmark distances or angles, or by principal components from outline data or landmark configurations.

For a correct cephalometric study we purpose ten measurements for the cranium, and seven for the mandible. All these measurements are distances between recognizable landmarks on digitalized images of the *normae dorsalis*, *basalis* and *lateralis* of the skull. The measurements

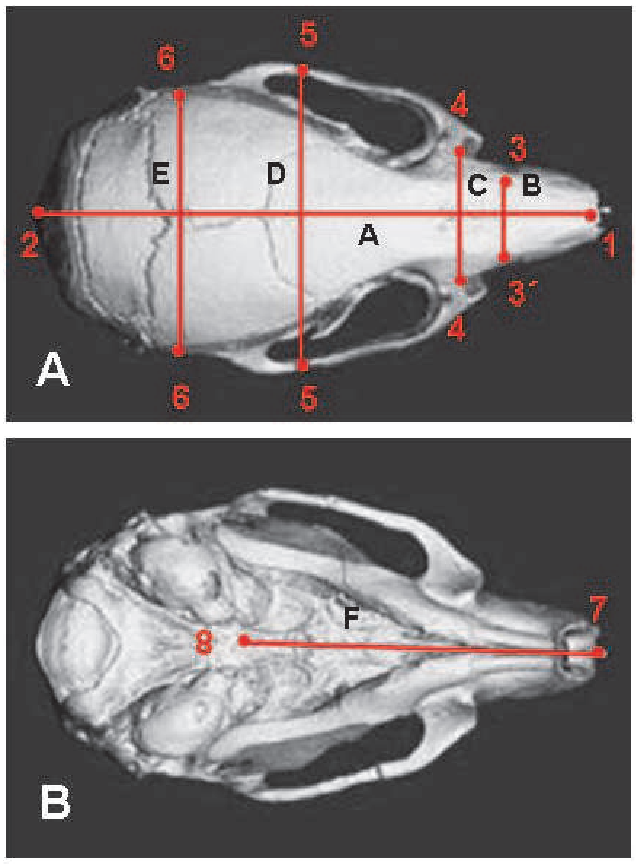

Fig. 2. Landmarks and measurements proposed for cephalometry in mice. A – Norma superior: 1: internasal point; 2: occipital point; 3: nasal points; 4: orbital point (right and left infraorbital foramina); 5: zygomatic points; 6: jugal process of squamosal bone; A: cranial length; B: internasal distance; C: interorbitary length; D: interzygomatic distance; E: bitemporal distance. B – Norma basalis: 7: interdental point; 8: posterior nasal spine; F: palatine length.

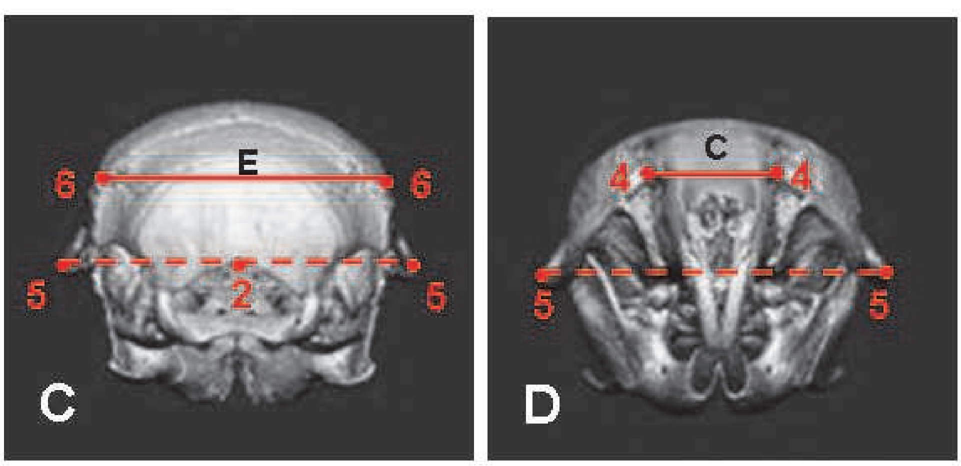

Fig. 2. Landmarks and measurements proposed for cephalometry in mice. C – Norma posterior: 2: occipital point; 5: zygomatic points; 6: jugal process of squamosal bone; E: bitemporal distance. D – Norma anterior: 4: orbital point (right and left infraorbital foramina); 5: zygomatic points; C: interorbitary length.

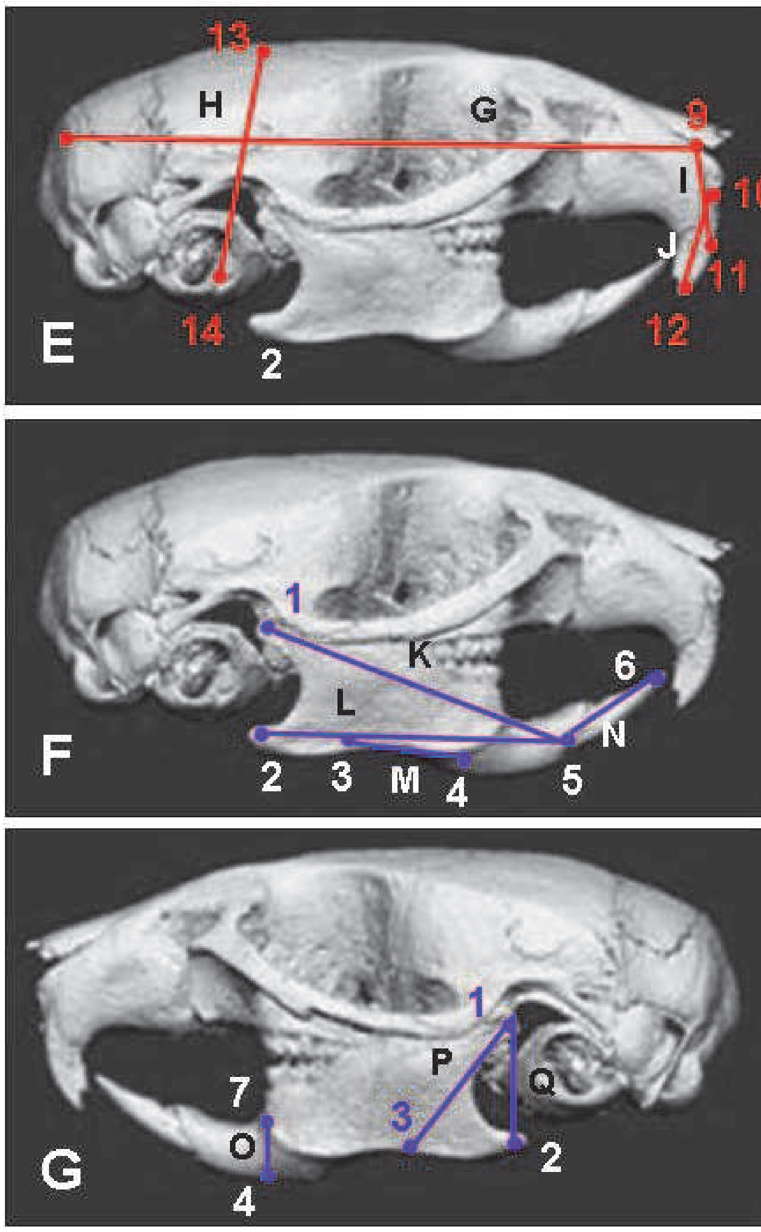

Fig. 2. Landmarks and measurements proposed for cephalometry in mice. E – Norma lateralis dextra: 9: naso-maxillary point; 10: superior incisor-alveolar point; 11: prostion; 12: superior incisor point; 13: parietal point; 14: tympanic point; G: sagittal cranial distance; H: posterior cranial height; I: anterior cranial height; J: upper incisor height. F – Norma lateralis dextra (mandible measurements): 1: condilion point; 2: gonion; 3: antegonion; 4: menton; 5: inferior incisor-alveolar point; 6: incisor inferior point; K: effective mandible length; L: mandible plain; M: mandible axis; N: inferior incisor axis. G – Norma lateralis sinistra (mandible measurements): 1: condilion point; 2: gonion; 3: antegonion; 4: menton; 7: mandible alveolar (or diastema) point; O: anterior mandible height; P: condilar axis; Q: posterior mandible height.

we purpose are based on other studies carried out in mice showing skull phenotypes caused by gene mutation (see Olafsdottir et al., 2007), and were homologous to those used for standard orthodontic cephalometry in humans (Burkhardt et al., 2003).

Accuracy of measurements should be a primary goal of scientists to prevent statistical errors and therefore to promote the comparison of the results obtained from various research groups. Therefore they must be vigilant during data collection and use the appropriate device/method. Skull measurements in mice require an accurate localization of landmarks and measurements, since errors can lead to inappropriate valuation of an experimental situation. The accuracy of cephalometric landmark identification it is not related to technical characteristic of the used 3D-µCT (Olszewski et al., 2008) but rather with the ability and training of the researchers. Moreover, 3D imaging allows for overall improved interobserver and intraobserver reliability in certain landmarks in vivo when compared with two-dimensional images, and intraexaminer and interexaminer reliabilities are high for most landmarks (Chien et al., 2009).

The following parameters are proposed (Figure 2):

Craniometric measurements:

1. Cranial length (A): measured between the internasal (top of the nose) and the occipital (the most distal point of the occipital bone) points.
2. Inter-nasal distance (B): measured between both nasal lateral points.
3. Inter-orbitary length (C): measured between right and left infraorbital foramina.
4. Inter-zygomatic distance (D): measured between both zygion points.
5. Bi-temporal distance (E): measured in the more distant point of the jugal process off squamosal with respect to the sagittal plane.
6. Sagittal cranial distance (G): measured between the occipital and the naso-maxillary point.
7. Posterior cranial height (H): measured between the tympanic and the parietal point.
8. Anterior cranial height (I): measured between the upper incisor and the prostion points.
9. Upper incisor height (J): measured between the upper incisor-alveolar bone and upper incisor edge.
10. Palatine length (F): measured between the posterior nasal spine and the inter-dental point.

Mandible measurements:

1. Posterior mandible height (Q): measured between the gonion and condilion points.
2. Condiloid axis (length of the ascending ramus) (P): measured between the condilion and antegonion points.
3. Anterior mandible height (O): measured between the menton and the mandibular alveolar (or diastema) points.
4. Effective mandible length (K): measured between the lower alveolar incisor (infradentale) and the condilyon points.
5. Mandible plain (L): measured between the gonion and the lower incisor-alveolar bone.
6. Mandible axis (M): measured between the antegonium and menton points.
7. Inferior incisor height (N): measured between the lower incisor-alveolar bone and the lower edge.

This method consent a complete quantitative evaluation of the length, height and, in a lesser extent, width of the skull. The results of the measurements we have performed in adult C57B1/6 mice using 3D-µC are summarized in table 1. In comparing these values with those obtained using simple radiography it can be observed that they are almost identical. However, some key measurements cannot be performed using plane radiography because landmarks cannot not be precisely localized (see table 1), thus reinforcing the usefulness of 3D-µCT in these studies. On the other hand, some measurements that may be of interest (i.e. Inter-molar maxillary distance, hemi-mandible length or inter-molar mandible length; see de Carlos et al., 2008) can be performed only if the mandible is isolated and detached off the skull.

Cranial measurements	µCT	SimpleRX
Line A: CL	22,61 ± 0,31	22,44 ± 0,46
Line B: Internasal D	3,85 ± 0,13	n.d
Line C: Inter-orbitary L	4,21 ± 1,1	3,96 ± 0,12
Line D: Interzygomatic D	12,12 ± 0,30	11,96 ± 0,22
Line E: Bi-temporal D	10,31 ± 0,17	10,41 ± 0,16
Line F: Palatine L	14,03 ± 0,11	n.d
Line G: Sagittal CD	21,22 ± 0,41	21,33 ± 0,42
Line H: Posterior CH	10,31 ± 0,21	10,04 ± 0,40
Line I: Anterior CH	2,69 ± 0,11	2,67 ± 0,16
Line J: Upper incisor H	4,02 ± 0,18	3,99 ± 0,16
Mandible measurements		
Line K: Effective ML	11,21 ± 0,20	n.d
Line L: M plain	10,39 ± 0,71	n.d
Line M: M axis	5,32 ± 0,33	n.d
Line N: Inferior incisor axis	4,30 ± 0,11	4,15 ± 0,21
Line O: Anterior MH	2,09 ± 0,09	2,09 ± 0,12
Line P: Condiloid axis	5,18 ± 0,10	n.d
Line Q: Posterior MH	4,13 ± 0,16	n.d

C = cranial; D = distance; H = height; L = length; M = mandible
n.d: not done

Table 1. Results of the cranium and mandible measurements in the mouse using µCT and simple radiography. Data were obtained from 10 adult C57B1/6 mice

So, the values of measurements of the mice skull on conventional radiographs are comparable with measurements on 3D-µCT, but 3D-µCT allows for the demonstration of structures and landmarks that are impossible to identify by conventional radiographic methods.
A computational atlas of the mice skull using 3D-µCT has been developed by Olafsdottir et al. (2009) to automatically asses the variations in skull morphology and size of a mice model of Crouzon's syndrome. Although this atlas is a powerful method due to its plasticity and the results obtained with this system are the measurements they perform (skull length, height and width and interorbital distance) are not sufficient to completely evaluate the skull, since the mandible is not considered, and there are gene mutations that specifically affect to this bone.

4. Advantages and disadvantages of µCT for cephalometric measurements in mice

In the 1970 decade clinical imaging was radically changed by the introduction of computed tomography (CT). Until then, the examination of small animals in research, especially of mice and rats, was limited by the resolving capacity of clinical CT scanners (see central image of figure 1). However, over the past three decades 3D-µCT imaging has rapidly advanced with higher quality spatial and temporal resolution, the introduction of the cone beam reconstruction algorithm, and the availability of scanners specific for non-invasive small animal imaging research. These technical advancements have allowed researchers to capture detailed anatomical images and precisely localize landmarks (see Cavanaugh et al., 2004; Nalçaci et al., 2010; Schambach et al., 2010).

The limitations of plain film radiographs in skull evaluation are well documented in different classical texts and the introduction of 3D visualization of the bony skeleton has been a breakthrough (Papadopoulos et al., 2002). There are numerous studies reporting that measurements obtained by 3D methods, especially μCT, are more reliable than the conventional method (see Ozsoy et al., 2009; Zamora et al., 2011). Nevertheless, in our hands both simple radiography and 3D-μCT offer similar results for most of the cranial measurements, but not for the mandible.

So, 3D-μCT is actually the best method for noninvasive imaging of mouse cranial anatomy. The principle advantages of 3D-μCT technology for evaluation of the skull are: first, the ability to easily view and manipulate images in any plane; second, the ability to repeat the measure on the same individual animal over time; and third, the ability to minimize tissue and/or animal sacrifice. 3D-μCT however has the following main limitations: first, the image acquisition time is somewhat long; second, extensive hands-on data manipulation of the raw data is required before the final images can be rendered; third, it is expensive. But any case, surely this method is the present and the future.

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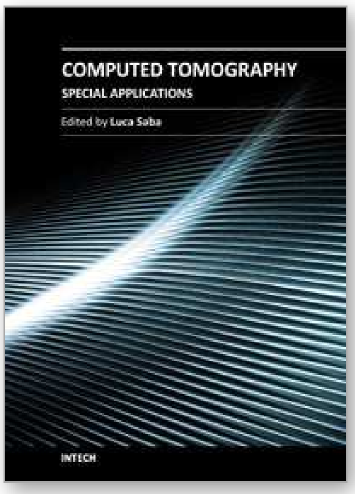

Computed Tomography - Special Applications
Edited by Dr. Luca Saba

ISBN 978-953-307-723-9
Hard cover, 318 pages
Publisher InTech
Published online 21, November, 2011
Published in print edition November, 2011

CT has evolved into an indispensable imaging method in clinical routine. The first generation of CT scanners developed in the 1970s and numerous innovations have improved the utility and application field of the CT, such as the introduction of helical systems that allowed the development of the "volumetric CT" concept. Recently interesting technical, anthropomorphic, forensic and archeological as well as paleontological applications of computed tomography have been developed. These applications further strengthen the method as a generic diagnostic tool for non destructive material testing and three dimensional visualization beyond its medical use.