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Three-dimensional growth pattern of the rat mandible revealed by periodic live micro-computed tomography

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A R T I C L E I N F O

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A B S T R A C T

Objective: We wanted to evaluate the three-dimensional (3D) mandibular growth of Sprague-Dawley rats from 4th to 16th postnatal weeks with periodic and live micro-computed tomographic scanning.

Design: Twenty Sprague-Dawley rats were used for micro-CT scanning from 4th to 16th postnatal weeks. After 3D reconstruction of rat mandible, we performed the linear and angular measurements and the superimposition of the 3D models to evaluate the mandibular growth of rat.

Results: The results showed that the growth direction of the condylar and coronoid regions was superior primarily and posterior secondarily, while the condyle had minimal lateral growth. Moreover, the angular region was growing mainly toward the posterior and lateral direction, while the body and symphysis maintained small, incremental anterior-posterior growth.

Conclusions: We could evaluate the amount, rate, and direction of growth using the mandibular skeletal unit. Some reference points and measurements were more relevant in properly characterizing 3D growth of the mandible. Their growth rates were the greatest between 4th and 8th postnatal weeks, a period which seems most appropriate for studies of rat mandible growth.

1. Introduction

Various animal models have been used for craniofacial morphometric growth studies (Losken, Mooney, & Siegel, 1992; Losken, Mooney, & Siegel, 1994; Sarnat, 1997). The rat is one such model due to the rat's diverse phylogeny, life cycle and reproductive capability (Sengupta, 2013). In addition, rats are generally known to present similar relative percent changes at the craniofacial regions to that of human being, especially during juvenile and adulthood stages (Losken et al., 1992). Thus much research has been dedicated to craniofacial growth in rats (Abed, Buschang, Taylor, & Hinton, 2007; Cleall, Jacobson, & Berker, 1971; Cleall, Wilson, & Garnett, 1968; Duterloo & Vilmann, 1978; Losken et al., 1992; VandeBerg, Buschang, & Hinton, 2004). Though we can derive insight into human craniofacial growth from experimental rat results, most rat studies to date have been based on two-dimensional (2D) images. Most of these studies have simply measured height or length for growth assessment. These data naturally set limits on the evaluation of three-dimensional (3D) spatial growth patterns of the craniofacial region. Standardized radiographic images such as cephalograms are one solution, but these are essentially 2D, making it difficult to model growth patterns accurately, as well as complicated to apply due to the limited head positioning (Cleall et al., 1968).

Recent studies have begun to utilize 3D images to analyze craniofacial structures or masticatory function in 3D space quantitatively and qualitatively (Kuroda et al., 2011; Mavropoulos, Kiliaridis, Bresin, & Ammann, 2004; Nakano, Maki, Shibasaki, & Miller, 2004). However, 3D morphometric studies of craniofacial growth have so far been based mainly on direct anthropometric measurements of skull or x-ray after animal sacrifice (Asano, 1986; Kiliaridis, Engstrom, & Thilander, 1985; Ulgen, Baran, Kaya, & Karadede, 1997). In addition, 3D reference points and measurements basically imitated those of traditional 2D works (Kim et al., 2008; Nakano et al., 2004).

The mandible, essential to masticatory function and facial morphology, is a simple structure articulated to the cranial base. In addition, the mandible is an important factor in total facial growth. Thus we

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wanted to evaluate the 3D growth of rat mandible by examining it in terms of our considered reference points and measurements and then comparing the results with periodic 3D image data produced by micro-CT. The mandible was divided on the basis of development, growth and function into several parts or units. Specifically, we focused on the mandible as an independent skeletal unit for periodic growth comparison with 3D micro-CT images (Moss & Young, 1960).

To achieve this goal, we reviewed the traditional mandibular reference points and measurements reported for rats (Abed et al., 2007; Cleall et al., 1971, 1968; Duterloo & Vilmann, 1978; Giglio, Lazzari, & Rebok, 1998; Losken et al., 1992; Moore, 1973; Ulgen et al., 1997; VandeBerg et al., 2004), performed periodic live imaging with micro-CT, and measured the mandibular structure with reference points for 3D image models. We aimed not just simply to measure the length, but to analyze the growth rate, direction, and amount of the skeletal units by time periods. These results will increase our understanding of the mandibular growth pattern in 3D and provide a sound basis for analyzing rat mandibular growth in further studies.

2. Materials and methods

2.1. Samples and data acquisition

Twenty male Sprague-Dawley rats (Orientbio Co., Ltd. Seongnam-si, Gyeonggi-do, Korea) at the age of postnatal 3 weeks were kept in quarantine for a week. The experiment was approved by a committee (Institutional Animal Care and Use Committee, Yonsei University Health System, Seoul, Korea) to meet the requirements of the Association for Assessment and Accreditation of Laboratory Animal Care International.

The rats were studied from postnatal 4th to 16th weeks while raised under normal conditions. This period corresponds to adolescence and young adulthood in humans. In-vivo micro-CTs were taken in a monitored anesthetic condition (NFR-Polaris-G90MVC, Nano Focus Ray, Kwangju, Korea) at the 4th, 8th and 12th week of age. To prevent motion artifacts, animals were anesthetized via intraperitoneal injection with Rompun (Bayer, Leverkusen, Germany) and Zoletile (Virbac, Carros, France). The micro-CT was set to 65 kV, 115 μA, pixel size 77 μm and slice thickness of 170.6 μm. At the end of the experiment, the rat was sacrificed according to ethical guidelines and the head part then scanned at higher resolution (17 μm) with another micro-CT system (Skyscan1076, Bruker, Konitch, Belgium).

The micro-CT data were stored in the Digital Imaging and Communications in Medicine (DICOM) format and 3D reconstruction of mandible and its analysis was performed with software (Simplant Pro, Materialise Dental Co., Leuven, Belgium; Mimics 17, Materialise Co., Leuven, Belgium).

2.2. Morphometric reference points and measurements

We reviewed previous studies, particularly focusing on reference points relevant to 3D mandibular growth and measurement (Abed et al., 2007; Cleall et al., 1971, 1968; Duterloo & Vilmann, 1978; Giglio et al., 1998; Losken et al., 1992; Moore, 1973; Ödman & Kiliaridis, 2010; Ulgen et al., 1997; VandeBerg et al., 2004). After a critical review, the selected reference points and 3D measurements were adopted as follows (Fig. 1A, Table 1):

1 The skeletal units

Following functional matrix and skeletal unit analysis (Moss, 1968a, 1969; Moss & Meehan, 1970; Moss & Young, 1960), the mandible was divided into five skeletal units, as previously reported (Giglio et al., 1998; Moore, 1973; Ödman & Kiliaridis, 2010; Park, 2014). Each unit was designed to represent developmental characteristics of the mandible. The skeletal unit at each interval was measured from the mandibular reference points (Fig. 1A; Table 1), including inferior alveolar foramen (IAF), mental foramen (MF), condyle (Con), coronoid (Cor), gonion (Go) and lower incisor alveolar point (L1(i)), these yielding the condylar unit (IAF-Con), coronoid unit (IAF-Cor), angular unit (IAF-Go), body unit (IAF-MF), and symphyseal unit (MF-L1(i)) (Fig. 2A; Table 2). Con was specially constructed on the point of the condylar head surface met by a line from IAF to the midpoint of right and left lateral condylar poles (as shown in Fig. 1B).

2 Vertical height, anterior-posterior length and transverse width

The vertical growth of mandible was evaluated by measuring the distance to each reference point from a constructed plane, the mandibular inferior border plane (IBP) (Figs. 1D and 2B; Table 2). The IBP was constructed to pass through menton (Me) and gonion tangent (Go (t)), while being perpendicular to the mandibular ramal plane, which runs parallel to the mandibular ramus by passing through Con-Go(t)-Me (Fig. 1D1 and D2).

The anterior-posterior growth of mandible was evaluated by measuring the distance from L1(i) to Con, Cor and Go (Fig. 2C; Table 2). The mandibular width was measured by the distance between the bilateral reference points of Con, Cor, Go, Go(t), MF, M1(a) and IAF (Fig. 2D; Table 2).

3 Angular measurements

The growth of mandibular ramus, condyle and inferior border was also studied by angular measurements. Additional reference planes were introduced for angular measurements (Fig. 1C): (1) the mandibular median plane as a reference sagittal plane, running through the midpoint of right and left IAF and MF, and perpendicular to the mandibular body plane (both IAF-midpoints of MF); (2) the mandibular axial plane as a reference axial plane, passing through the midpoint of right and left Con, being parallel to the Frankfort horizontal plane (both porions and midpoint of both orbitales) and perpendicular to mandibular median plane; (3) the mandibular coronal plane as a reference coronal plane, passing through the midpoint of right and left Con, and perpendicular to both mandibular median plane and mandibular axial plane. We set the line for the ramal axis (Con-Go(t)), condylar unit (Con-IAF), mandibular axis (Con-L1(i)), and mandibular inferior border (Me-Go(t)) and measured the angles between each line and mandibular median plane or IBP, which were projected onto the coronal, sagittal, and axial plane respectively (Fig. 2E–G; Table 2).

2.3. Assessment of mandibular interval growth

The absolute and relative growth rate of the mandible were analyzed by measuring the length and angles, and by comparing interval changes at T0 (4th week of age), T1 (8th week), T2 (12th week) and T3 (16th week). The relative growth rate was evaluated by calculating the relative ratio of each measurement to that of T0, and the absolute growth rate was done by comparing measurements from each time point (Losken et al., 1992). This method of comparison was specifically useful for comparing the samples with individual variations. In order to visualize the chronologic dimensional changes of the mandible, the 3D reconstructed mandibular models of the four stages were superimposed based on the registration at IAF and MF, as previously suggested (Ödman & Kiliaridis, 2010) (Fig. 3A–F).

2.4. Statistics and methods error

Statistical comparisons of mean values and variance were produced using repeated measures analysis of variance (RM ANOVA) and post hoc Turkey test using Statistical Package for the Social Sciences (SPSS, Version 21, IBM Co.). Reference point errors on the 3D models were evaluated by digitizing the points repeatedly 20 times for 5 points and