学 位 論 文 の 要 旨

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学 位 論 文 名　Mathematical Analysis of Mandibular Morphogenesis by Micro-CT-Based Mouse and Alizarin Red S-Stained-Based Human Studies During Development

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論 文 内 容 の 要 旨

INTRODUCTION

Mandible consists of two symmetrical halves (right and left), each formed by a dentary bone with four morphogenetic regions: the mandibular body (ramus) and three processes (coronoid, condylar, and angular). Establishing normative expectations for experimentally induced changes in size and shape will be an important innovation in three-dimensional (3-D) micro-computed tomography (CT)-based morphological assessments of developing mandibles. Micro-CT has been adapted to the 3-D visualization of embryonic morphology, and can play a prominent role in quantitative studies of mandibular growth and development. A relatively recent technique, high-resolution micro-CT combined with morphometric analyses allows evaluation of the mandible and determination of shape changes in bone morphology. These methods were developed to analyze the differences in shape among samples based on anatomic landmarks and can give a quantitative account of shape-change; they have been used successfully in biological and clinical comparisons. Morphological studies of subtle anomalies in the field of morphogenesis and tests for the developmental toxicity of new drugs, pesticides, or food additives have focused mainly on abnormal shape, that is, maldevelopment of bone and other parts. The majority of studies have focused on postnatal ontogenetic patterns, but an understanding of the ontogenetic pattern during the prenatal period is of particular importance because distinctive morphogenetic divergence is more concentrated at this stage. In this study, mathematical analyses were performed of not only the normal prenatal and postnatal mouse mandible but also those of mechanically stressed or genetically altered mice to test and develop a

sensitive method to detect slight deviation from normal development. We also studied the developmental process of the human mandible in the fetal period.

MATERIALS AND METHODS

Jcl:ICR female mice were used. After pregnant mice were deeply anesthetized with di-ethyl ether, fetuses from embryonic day (E) 14.5 to E18.5 were obtained. Postnatal mice [postnatal day (P) 1 to P14, P21, and P28] were sacrificed by decapitation under ether anesthesia. The heads of the postnatal mice and bodies of the fetuses were fixed with 10% formalin in 70% methanol solution, dehydrated with 70% ethanol before performing CT scan. At E15.5, *exo utero* surgery was performed to restrict temporomandibular joint (TMJ) and the fetuses' mandible and maxilla were fixed by an 8-0 nylon suture, sham-operation was also performed by passing the needle from the mandible through the maxilla without making a knot, and were allowed to develop *exo utero* till E18.5. Receptor tyrosine kinase-like orphan receptor (Ror) 2 mutant heterozygous ($Ror2^{+/-}$) C57BL/6J mice were crossed and E15.5 and E18.5 fetuses were obtained. $Ror2^{+/+}$, $Ror2^{+/-}$, and $Ror2^{-/-}$ fetuses were identified by polymerase chain reaction (PCR) genotyping. Human fetuses were from the Kyoto collection, Kyoto University, and were fixed in 10% formalin solution after abortion and preserved in glycerol with ethanol in equal proportion (1:1). The fixed specimens were dehydrated by 70% ethanol and were stained by alizarin red S. The entire CT scanned images and photo images of bone-stained samples were transferred to a personal computer for mathematical analyses, that is, the thin-plate spline (TPS) analysis and principal warps analysis. The right hemi-mandible of each specimen was taken for analysis and we digitized some anatomical landmarks, which we assumed to be homologous among different samples. TPS analysis describes shape change by interpolating between the relative displacements of discrete landmarks and produces a rigorous quantitative analysis of the spatial organization of shape change by bending energy (BE) and Procrustes distance (PD). Principal warps are the eigenfunctions of the BE matrix interpreted as actual warped surfaces over the surface of the original landmark configuration, and include partial and relative warps.

RESULTS AND DISCUSSION

Morphogenesis of the mouse mandible during development revealed that the BE and PD were larger in the prenatal period than in the postnatal period especially after P3, whereas the volume of the mandible increased rapidly in the postnatal stages. These findings indicate that significant modifications in the shape of the mandible take place in the prenatal stages of mouse development.

Prenatal TMJ movement restriction using the *exo utero* development system is a strong mechanical stimulation to produce disharmony in the mandibular development. This restriction

of jaw movement led to the morphological deformation of the mandible in the E18.5 sutured fetuses, and we observed the deformation by using CT scan and 3-D reconstruction, compared with those in the sham-operated group at E18.5. The sutured mandible was shorter in length and thinner in width than those of the sham-operated mandible. Morphometric analysis revealed a significant increase in the PD in the sutured group. The partial and relative warp analyses showed a clear separation of the sutured group from the sham-operated group. TPS analysis has been found effective for detecting abnormality, which depends significantly on the development of the mandible. TPS analysis thus appears to be particularly efficient for the description and statistical evaluation of shape variations occurring during mandibular development.

Ror2 has been demonstrated to play essential roles in developmental morphogenesis, and $Ror2^{-/-}$ mice exhibit short snouts. Thus, the $Ror2^{-/-}$ mouse mandibles were expected to be malformed. The present CT scan and 3-D reconstruction of the mandible clearly demonstrated altered mandibles in the $Ror2^{-/-}$ group at E18.5 from those in the $Ror2^{+/+}$ and $Ror2^{+/-}$ groups. Volume was significantly reduced in the $Ror2^{-/-}$ mandibles compared to the $Ror2^{+/+}$ and $Ror2^{+/-}$ mandibles at both E15.5 and E18.5. The volume of $Ror2^{+/-}$ mandibles was also significantly reduced compared to the $Ror2^{+/+}$ mandibles at E18.5. In contrast, BE and PD both were significantly increased in the $Ror2^{-/-}$ mandibles compared with $Ror2^{+/+}$ and $Ror2^{+/-}$ mandibles at E18.5, while there were no significant differences in the BE and PD among them at E15.5, probably because the mineralization process was still at the early stage. The ordination scatter plot by principal warps showed that $Ror2^{-/-}$ mandibles were highly divergent from $Ror2^{+/+}$ and $Ror2^{+/-}$ mandibles at E18.5, and $Ror2^{+/+}$ and $Ror2^{+/-}$ groups had very similar morphological characteristics. These findings suggest that these methods are very sensitive and specific at detecting abnormal development of the mandible during fetal development.

Evaluation of the development of the human mandible is one of the most potent tools for studying the clinical orientation of the mandible, for example, facial asymmetry, malocclusion etc. Clinical observation has suggested that midfacial profiles are established early in fetal development and are maintained postnatally. We found in the bone-stained mandibles that the rate of BE was higher in 121-152 mm crown-rump length (CRL) than in those of the other weeks, whereas the rate of PD did not show any peak. The results of this study are important to clinicians' ability to detect the abnormal development of the mandible prenatally for early intervention to minimize functional impairment.

CONCLUSION

Our study shows that these mathematical methods are effective for elucidating the morphological development of the mandible as well as for detecting abnormality during development.