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Morphology and morphometry of the human embryonic brain: A three-dimensional analysis

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ABSTRACT

The three-dimensional dynamics and morphology of the human embryonic brain have not been previously analyzed using modern imaging techniques. The morphogenesis of the cerebral vesicles and ventricles was analyzed using images derived from human embryo specimens from the Kyoto Collection, which were acquired with a magnetic resonance microscope equipped with a 2.35-T superconducting magnet. A total of 101 embryos between Carnegie stages (CS) 13 and 23, without apparent morphological damage or torsion in the brain ventricles and axes, were studied. To estimate the uneven development of the cerebral vesicles, the volumes of the whole embryo and brain, prosencephalon, mesencephalon, and rhombencephalon with their respective ventricles were measured using image analyzing Amira™ software. The brain volume, excluding the ventricles (brain tissue), was 1.15 ± 0.43 mm^3 (mean ± SD) at CS13 and increased exponentially to 189.10 ± 36.91 mm^3 at CS23, a 164.4-fold increase, which is consistent with the observed morphological changes. The mean volume of the prosencephalon was 0.26 ± 0.15 mm^3 at CS13. The volume increased exponentially until CS23, when it reached 110.99 ± 27.58 mm^3. The mean volumes of the mesencephalon and rhombencephalon were 0.20 ± 0.07 mm^3 and 0.69 ± 0.23 mm^3 at CS13, respectively; the volumes reached 21.86 ± 3.30 mm^3 and 56.45 ± 7.64 mm^3 at CS23, respectively. The ratio of the cerebellum to the rhombencephalon was approximately 7.2% at CS20, and increased to 12.8% at CS23. The ratio of the volume of the cerebral vesicles to that of the whole embryo remained nearly constant between CS15 and CS23 (11.6–15.5%). The non-uniform thickness of the brain tissue during development, which may indicate the differentiation of the brain, was visualized with surface color mapping by thickness. At CS23, the basal regions of the prosencephalon and rhombencephalon were thicker than the corresponding dorsal regions. The brain was further studied by the serial digital subtraction of layers of tissue from both the external and internal surfaces to visualize the core region (COR) of the thickening brain tissue. The COR, associated with the development of nuclei, became apparent after CS16; this was particularly visible in the prosencephalon. The anatomical positions of the COR were mostly consistent with the formation of the basal ganglia, thalamus, and pyramidal tract. This was confirmed through comparisons with serial histological sections of the human embryonic brain. The approach used in this study may be suitable as a convenient alternative method for estimating the development and differentiation of the neural ganglia and tracts. These findings contribute to a better understanding of brain and cerebral ventricle development.

Introduction

Originating from a simple neural tube, the brain becomes an elaborate structure through a series of differentiation processes (O'Rahilly and Müller, 2006; Bayer and Altman, 2008; Huang et al., 2009). The three brain vesicles that form at the cranial end of the neural tube differentiate to form the prosencephalon, mesencephalon, and rhombencephalon at Carnegie stage (CS) 13 (O'Rahilly and Müller, 1987). CS is a standardized system of 23 stages used to provide a unified developmental chronology of the human embryo. The stages are delineated through the development of external and internal structures, not by size or the number of days of development. The brain achieves its definitive organization after CS15 with the growth of the telencephalon. The diencephalon becomes enclosed between the cerebral hemispheres on both sides.

Classically, three-dimensional (3D) developmental anatomy was analyzed with serial histological sections and visualized with 3D modeling

Abbreviations: CS, Carnegie stage; LV, lateral ventricle; MR, magnetic resonance; 3D, three-dimensional; COR, core region.

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and/or illustrations. Those methods were laborious and inaccurate in the translation of two-dimensional histology into a 3D object. Recent advances in magnetic resonance (MR) imaging, computed tomography (CT), and 3D sonography have provided 3D digital information, which has been applied to clinical diagnosis. In the field of fetal medicine, 3D images of the fetal brain acquired with MR imaging during the second trimester have been studied (Kinoshita et al., 2001; Huang et al., 2009); however, 3D imaging during the embryonic period proper (the first 2 months of the first trimester) have not been fully analyzed. In the present study, the morphogenesis of the human embryonic brain was analyzed via 3D reconstructions from MR microscopic data. The data provided demonstrates the dramatic growth of the human brain during the embryonic period in each CS.

Materials and methods

Human embryo specimens

Approximately 44,000 human embryos comprising the Kyoto Collection are stored at the Congenital Anomaly Research Center of Kyoto University (Nishimura et al., 1968; Shiota, 1991; Yamada et al., 2006; Shiraishi et al., 2013). In most of these cases, pregnancy was terminated during the first trimester for socioeconomic reasons under the Maternity Protection Law of Japan. Approximately 20% of the embryos were not damaged and were well preserved. In the laboratory, aborted embryos were measured, examined, and staged using the criteria provided by O'Rahilly and Müller (1987). Approximately 1200 well-preserved human embryos found by two of the authors (C.U. and S. Y.) to be normal on gross examination and between CS13 and CS23 were selected for MR microscopic imaging. The conditions used to acquire the MR images of the embryos have been previously described elsewhere (Matsuda et al., 2003, 2007; Yamada et al., 2006; Shiota et al., 2007). Briefly, The MR images of the embryos were acquired using a super-parallel MR microscope developed with a 2.35 T horizontal bore (40 cm) superconducting magnet (Matsuda et al., 2007). The pulse sequences used for the image acquisition were T_1-weighted spin echo sequences with 100 ms repetition times and 10–16 ms echo times. The image matrix was $128 \times 128 \times 256$ and the size of the voxel varied from $(40\ \mu m)^3$ to $(150\ \mu m)^3$. Because the number of signal accumulations was 16 or 24, the total data-acquisition time was 7.3 or 10.9 h. As shown in the previous paper (Matsuda et al., 2007), the image intensity of the T_1-weighted images of the human embryos has a close correlation with that of Nissl staining sections.

For the present study, 101 samples at different CS between CS13 and CS23 (9 or 10 samples for each stage, except CS13, for which there were 5 samples) were selected from the 1200 MR image datasets for 3D reconstruction and morphometric analysis. The selected embryos were re-examined by two authors (T.N. and T.T.) based on previously described criteria (Nakashima et al., 2012). The samples with apparent deformity and brain shrinkage were excluded from the analysis because prolonged fixation is known to cause MRI artifacts and tissue shrinkage due to dehydration (van Duijn et al., 2011).

Three-dimensional reconstruction and morphometric analysis

Three-dimensional MR image datasets for each embryo were resectioned as sequential 2D images digitally with ImageJ64™ (ver. 1.44, National Institutes of Health, Bethesda, Maryland, United States) and saved as Analyze file formats (.hdr, .img). On MR imaging, brain tissue showed layered structures with high intensity signals. It was difficult to make precise distinctions between histological structures within the brain tissue, though the borders between brain tissues and the surrounding tissues, such as the subarachnoid spaces, ventricles, and mesenchymal tissues, were clear (data not shown).

The brains and ventricles were segmented for 3D reconstruction using FSL View of FMRIB Software Library™ (ver. 4.1.9, Analysis Group, FMRIB, Oxford, UK). Three-dimensional morphology of the brain was computationally reconstructed with Amira™ software (ver. 5.4.0, Visage Imaging, Berlin, Germany).

The regional non-uniform thickness of the brain tissue was visualized using the following two filter modules of the Amira™ software program: 1) surface thickness (the thickness of the brain was visualized on the surface with a color scale) and 2) extraction of the "core region." Layers of tissue were digitally subtracted from the brain tissue by equal amounts from both the external and internal surfaces. The remainder was visualized as the core region (COR) of the thickening brain tissue. The dorso-lateral part of the telencephalon (cortex) was used as a reference to determine the number of layers needed to be subtracted to isolate the COR in the respective samples.

The volumes of the brain and whole embryo were calculated using OsiriX™ software (ver. 4.0, Pixmeo SARL, Geneva, Switzerland). The brain vesicles were divided into three regions according to anatomic landmarks. The supramammillary recess and posterior commissure were used to define the prosencephalon and mesencephalon, the isthmic recess and the isthmic groove to define the mesencephalon and rhombencephalon, and the level of the C1 vertebra to define the separation between the rhombencephalon and spinal cord (Nakashima et al., 2012). The cerebellum was segmented using the Amira software program after visualization of the COR. The anatomical references used were the isthmic groove and the roof of the fourth ventricle.

This study was approved by the Committee of Medical Ethics of Kyoto University Graduate School of Medicine, Kyoto, Japan (E986).

Results

Morphogenesis of the reconstructed brain

All 101 brains between CS13 and CS23 were reconstructed for morphological and morphometric analysis. The 3D reconstruction allowed us to make precise gross observations (Fig. 1A, Supplementary File 1 in Shiraishi et al., submitted for publication). The three brain vesicles, formed at the cranial end of the neural tube, differentiated to form the prosencephalon, mesencephalon, and rhombencephalon at CS14. The telencephalon differentiated from the prosencephalon after CS15, and cerebral hemispheres became apparent on both sides of the diencephalon at CS17. The cerebral hemispheres grew in a pattern similar to that of a ram horn, with an arch directed backward and spirally outward (known as the rotation of the hemispheres) until CS23. Changes in internal morphology, including the formation of the ventricular system, were also recognizable (Fig. 1B). During the dynamic embryonic differentiation of the prosencephalon, the lateral ventricles (LVs) and the third ventricle formed, and the fourth ventricle developed in the rhombencephalon.

Morphometry of the reconstructed brain

The brain tissue volume and ventricular volume between CS13 and CS23 were calculated for all 101 reconstructions samples. The brain volume excluding the ventricles (brain tissue) was $1.15 \pm 0.43\ mm^3$ at CS13 and increased exponentially to $189.10 \pm 36.91\ mm^3$ at CS23 (Fig. 2). The brain tissue volume increased 164.4-fold, which is consistent with the observed morphological changes (Fig. 1A and B).

To assess brain growth during the embryonic period, the volume of brain tissue was compared to the whole embryo volume. The whole embryo volume was $12.73 \pm 3.63\ mm^3$ at CS13 and exponentially increased to $1453.84 \pm 418.05\ mm^3$ at CS23. The ratio of brain tissue volume to embryo volume was 9.0% at CS13 and increased to 13.1% at CS15. The ratio remained within a narrow range after CS15, between 11.6% (CS17) and 15.5% (CS22). The brain tissue volume expanded 34.7-fold, compared with a 34.9-fold expansion of the whole embryo volume, between CS15 and CS23. This data indicates that the growth rate of brain tissue and that of the whole embryo is comparable between CS15 and CS23.