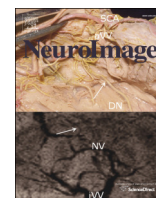


Morphology and morphometry of the
human embryonic brain:
A three-dimensional analysis

(ヒト胚子期における脳の三次元形態
計測学的解析)

白石 直樹



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 $1.15 \pm 0.43 \text{ mm}^3$ (mean $\pm$ SD) at CS13 and increased exponentially to $189.10 \pm 36.91 \text{ mm}^3$ at CS23, a 164.4-fold increase, which is consistent with the observed morphological changes. The mean volume of the prosencephalon was $0.26 \pm 0.15 \text{ mm}^3$ at CS13. The volume increased exponentially until CS23, when it reached $110.99 \pm 27.58 \text{ mm}^3$. The mean volumes of the mesencephalon and rhombencephalon were $0.20 \pm 0.07 \text{ mm}^3$ and $0.69 \pm 0.23 \text{ mm}^3$ at CS13, respectively; the volumes reached $21.86 \pm 3.30 \text{ mm}^3$ and $56.45 \pm 7.64 \text{ mm}^3$ 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.

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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 T₁-weighted spin echo sequences with 100 ms repetition times and 10–16 ms echo times. The image matrix was 128 × 128 × 256 and the size of the voxel varied from (40 μm)³ to (150 μm)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 T₁-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 isthmus recess and the isthmus 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 isthmus 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 $1.15 \pm 0.43 \text{ mm}^3$ at CS13 and increased exponentially to $189.10 \pm 36.91 \text{ mm}^3$ 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 $12.73 \pm 3.63 \text{ mm}^3$ at CS13 and exponentially increased to $1453.84 \pm 418.05 \text{ mm}^3$ 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.

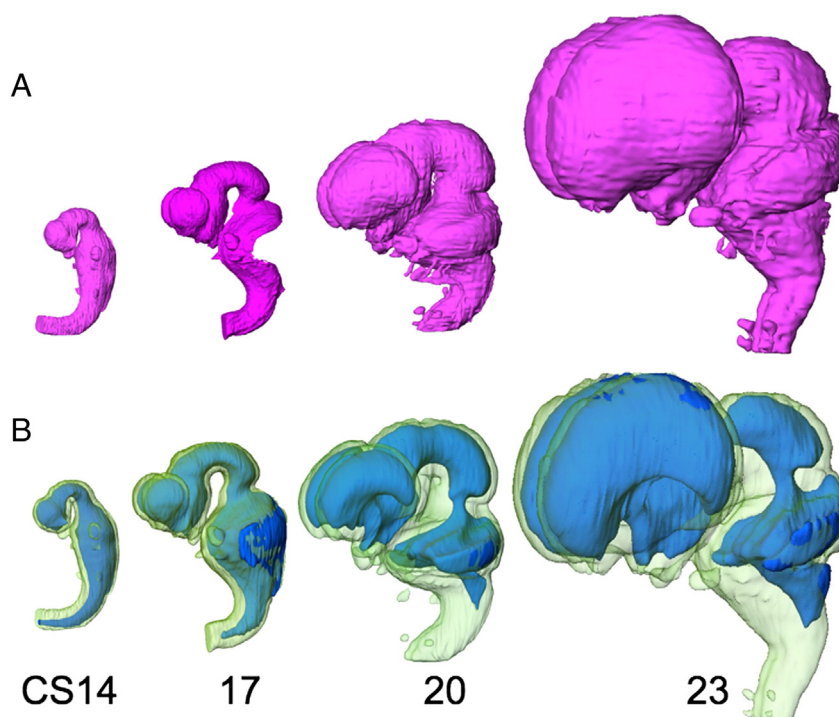


Fig. 1. Representative 3D images of the brain at CS14, CS17, CS20, and CS23. A) External view of the whole brain (same scale). B) Morphology of the ventricles observed through transparent brain tissue (same scale). Representative 3D movies of the brain using the same scale demonstrating the development and growth of the brain are shown in Supplementary File 1 in Shiraishi et al. (submitted for publication).

The tissue volume of the main three vesicles was also calculated. Though the diencephalon and telencephalon differentiated after CS15, the border of these two vesicles was not clearly discernible using the present MR data up to CS23. The tissue volume of the three brain vesicles grew exponentially. The brain tissue volumes of the prosencephalon, mesencephalon, and rhombencephalon were $0.26 \pm 0.15 \text{ mm}^3$, $0.20 \pm 0.065 \text{ mm}^3$, and $0.69 \pm 0.23 \text{ mm}^3$ at CS13, respectively. The volume increased exponentially, and reached $110.99 \pm 27.58 \text{ mm}^3$, $21.86 \pm 3.30 \text{ mm}^3$, and $56.45 \pm 7.64 \text{ mm}^3$ at CS23, respectively (Fig. 3). The tissue volume of the rhombencephalon was higher than

that of the prosencephalon between CS13 and CS19. However, the tissue volume of the prosencephalon was higher than that of the rhombencephalon after CS20 as the volume of the prosencephalon increased faster than that of the rhombencephalon.

The brain tissue volume was compared with the cavity volume. The internal cavities of the brain, namely the brain ventricles, were prominent especially in the early embryonic stages. The brain ventricle volume was $0.94 \pm 0.38 \text{ mm}^3$ at CS13 and increased to $97.25 \pm 28.29 \text{ mm}^3$ at CS23 (Fig. 4A). The volume of the cavities was greater than that of the brain tissue between CS14 and CS18. The ratio of the whole brain cavity volume to tissue volume reached a maximum at CS17 (1.46). After CS17, the volume of brain tissue was greater than

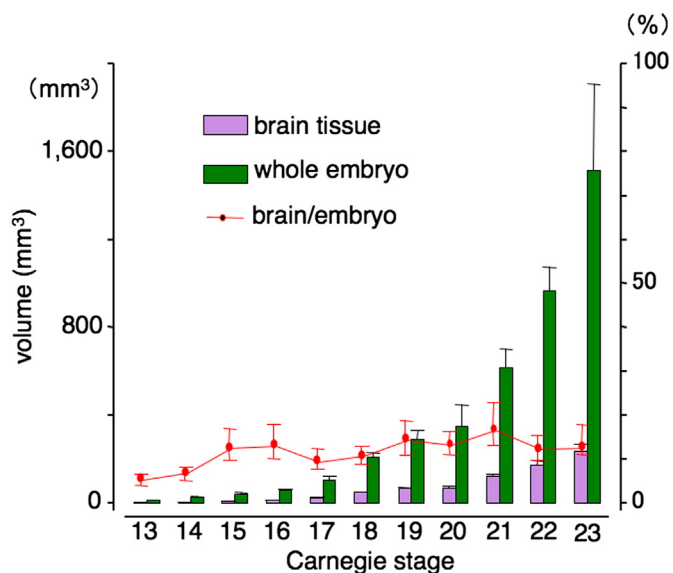


Fig. 2. Calculated volume of brain tissue and whole embryos between CS13 and CS23 [mean $\pm$ standard deviation (mm^3)].

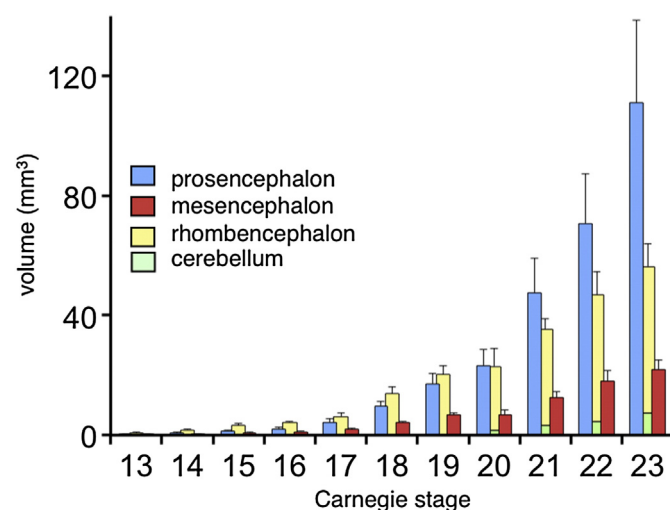


Fig. 3. Calculated volumes of the three brain vesicles without ventricles between CS13 and CS23 [mean $\pm$ standard deviation (mm^3)].

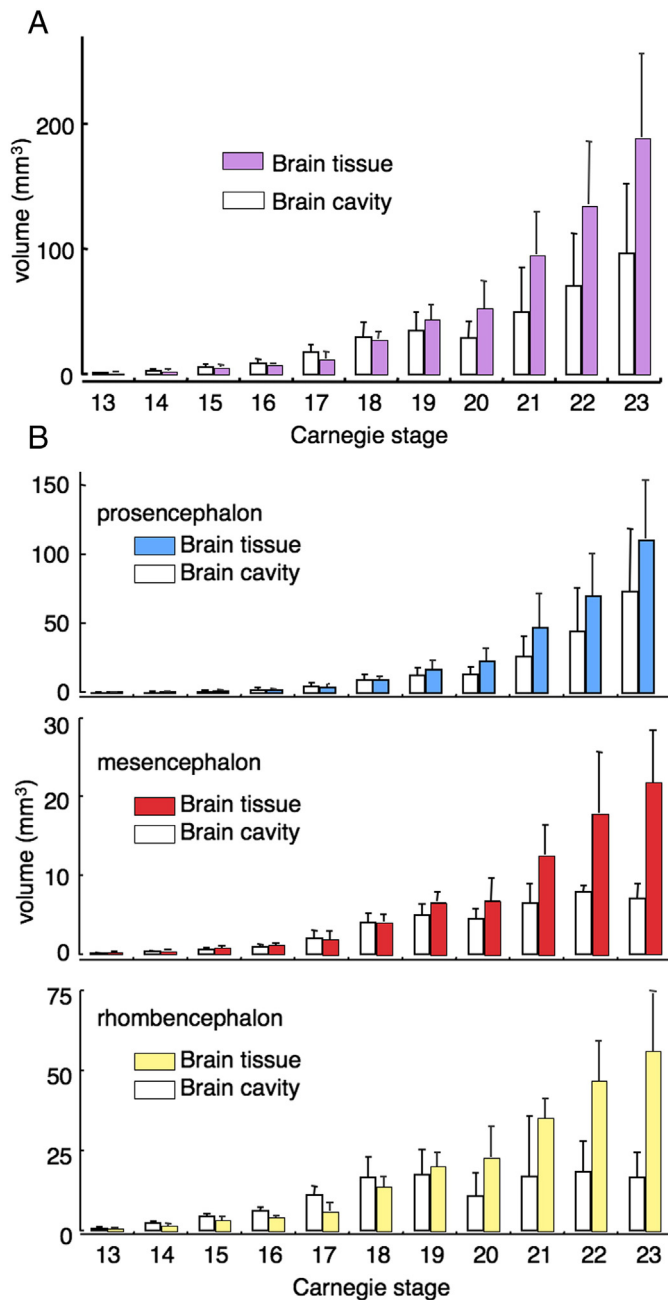


Fig. 4. Calculated volumes of the brain tissue and ventricles between CS13 and CS23 [mean $\pm$ standard deviation (mm³)]. (A) Comparative volumes of whole brain tissue and brain cavities. (B) Comparative volumes of individual vesicles and their respective cavities.

the brain cavity volume. The maximum brain cavity volume to brain tissue volume ratio was noted at CS17 in all three regions (prosencephalon: 1.11, mesencephalon: 1.04, and rhombencephalon: 1.84), and was particularly prominent in the rhombencephalon (Fig. 4B). This increase in brain cavity volume may result in an overestimation of brain growth.

The non-uniform thickness of brain tissue during development

As the brain develops, the thickness of brain tissue changes and contributes to the formation of this complicated organ. We visualized these dynamic changes in thickness on the brain surface using a color scale (Fig. 5, Supplementary File 2 in Shiraishi et al., submitted for

publication). The brain was relatively uniform in thickness between CS13 and CS16. However, non-uniformity in the thickness of the brain tissue was distinct after CS17. The ventricular eminences were detectable after CS17. The base of the telencephalon (basal ganglia) and the rhombencephalon, along with the primordial cerebellum become thickened, while the alar parts of the telencephalon, mesencephalon, and rhombencephalon remained thin.

The filter module use to serially subtract brain tissue from the external and internal surfaces of the developing brain enabled 3D visualization of the core regions of the thickening brain tissues resulting from brain differentiation and growth (Fig. 6). The COR correlated to the development of the nuclei and tracts and became apparent after CS17. This thickening was first seen in the basal parts of the rhombencephalon, cranial nerve nuclei, and the lateral and medial ventricular eminences in the prosencephalon. The anatomical positions of the COR were mostly consistent with nuclei such as the basal ganglia, thalamus, hypothalamus, pyramidal tract, and cranial nerve nuclei. The differentiation of the cerebellum was visualized as well. This was confirmed through a comparison with our own serial histological sections and an atlas of the human embryonic brain (Gasser et al., 2014; Bayer and Altman, 2008) (see Supplementary File 1 in Shiraishi et al., submitted for publication). A right-left difference regarding the increasing thickness of the brain tissue was not detectable in the present study.

The differentiation of the rhombencephalon is unique and complicated, and morphological descriptions are limited, especially with regard to the internal view and thickening of the brain tissue. The brain cavity of the rhombencephalon, the eventual fourth ventricle, did not grow exponentially and reached its maximum volume at CS19 (Fig. 4B). Nonuniformity in the thickness of the rhombencephalon tissue was visualized as external and internal cerebellar swelling, detectable after CS18 (Fig. 7, Supplementary File 3 in Shiraishi et al., submitted for publication). The ratio of the cerebellum to the rhombencephalon was approximately 7.2% at CS20, and increased to 12.8% at CS23 (Fig. 3).

Discussion

The dynamics of embryonic brain development have not been fully described. Most of the limited data available describe the initiation of fetal brain growth (Grenell and Scammon, 1943; Dunn, 1921). Jenkins (1921) measured the relative weight and volume of the component parts of the brain in 10 samples at different stages of development, including three samples from the embryonic period. With regard to the embryonic period proper, Desmond and O'Rahilly (1981) measured the major axes of the three embryonic brain vesicles and showed that the rates of growth of all three embryonic brain vesicles were much greater than the growth of the corresponding vesicles during the fetal period. Levitan and Desmond (2009) measured the areas of median sections two dimensionally to describe the growth of the three primary brain vesicles and to determine the change in the ratio of tissue area to cavity area. These studies were not able to accurately reconstruct the growth of the brain, region by region.

In the present study, we created a precise 3D reconstruction of the human embryonic brain with MR imaging data from 101 samples. We were able to accurately characterize the morphology and anatomy throughout the different stages of human embryonic brain development, improving our understanding of this highly ordered process.

Three-dimensional morphometry was possible after sectioning the regions. We were able to measure and analyze these digital sections to study the morphology and volume of the whole embryo and brain and the prosencephalon, mesencephalon, and rhombencephalon, with their respective ventricles. The present data may be applied as a standard for each stage, having been derived from a statistically adequate sample size.

The ratio of embryonic brain tissue volume to the whole embryo volume increased from CS13 to CS15, and then remained nearly

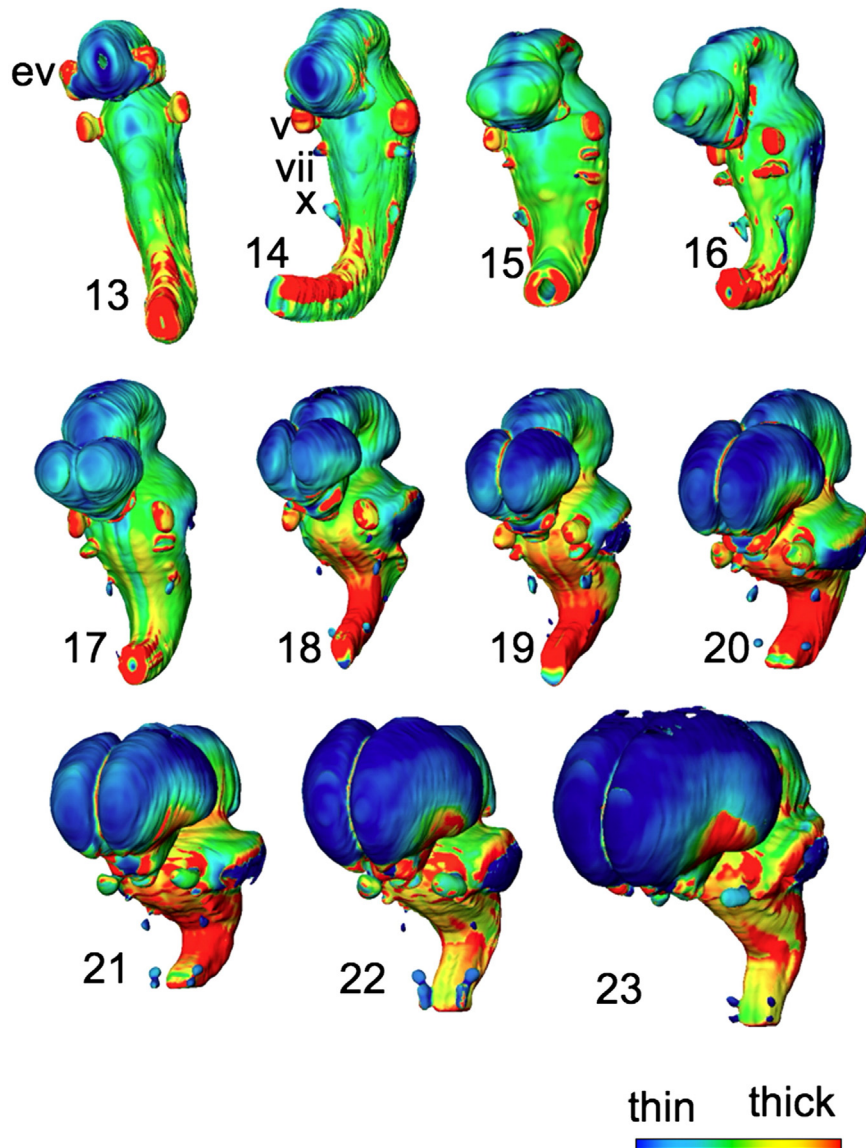


Fig. 5. Surface color mapping external view of the whole brain v, the trigeminal nerves; vii, the facial nerves; x, the vagus nerves; and ev, eye vesicles. Representative 3D movies of the brain for all stages between CS13 and CS23 are shown in Supplementary File 2 in Shiraishi et al. (submitted for publication).

constant between CS15 and CS23. Levitan and Desmond (2009) previously noted that an increase in the volumes of the brain cavities is a feature of the embryonic period. The cavity volume was relatively large, but there was no exponential increase in cavity volume, despite the complicated changes that the cavity underwent in the late embryonic stages in our study. The brain demonstrates conspicuous growth during the embryonic periods, which in a large part is the result of an expansion of the brain cavity. This increase in cavity volume may result in an overestimate of the growth rate of the brain. During the period of organogenesis, almost all organs other than the brain simultaneously develop at maximum speed. The present data indicate that the growth rate of the brain tissue alone is not as rapid during organogenesis, except perhaps in the earlier stages between CS13 and CS15.

Morphometry of the three major divisions of the embryonic brain clarified the features of the individual brain vesicles. The present study showed that the morphology of both the brain tissue and cavity of the rhombencephalon was affected by the development of the pons and medulla of the brain stem and the cerebellum according to their respective growth time lines. Previous studies have suggested that morphometric

changes of the rhombencephalon differ from the other vesicles (Desmond and O'Rahilly, 1981; Levitan and Desmond, 2009). In our study, growth of the brain stem was apparent until CS17, prior to detectable growth of the cerebellum. External and internal cerebellar swellings were detectable after CS18. This complicated morphogenesis is responsible for the morphometric changes. The volume of the tissue and cavity increased in a non-exponential fashion and cavity volume reached a maximum at CS19.

In contrast to previous studies (Desmond and O'Rahilly, 1981), the growth rate of the rhombencephalon exceeded that of the prosencephalon until CS19. Morphological analysis in the present study revealed that the ventral part of the rhombencephalon thickened, corresponding to growth of the brain stem and neural tracts. In contrast, the prosencephalon appeared larger with the emergence of the cerebral hemispheres on both sides, but the tissues of these structures remained thin during the embryonic period. After CS20, the growth of the prosencephalon becomes much greater than that of the other 2 sections, as the cerebral hemispheres grow rapidly, contributing to the exponential growth of the brain observed during the fetal period. The rate of growth of the prosencephalon continues to be greater than that of the rest of the

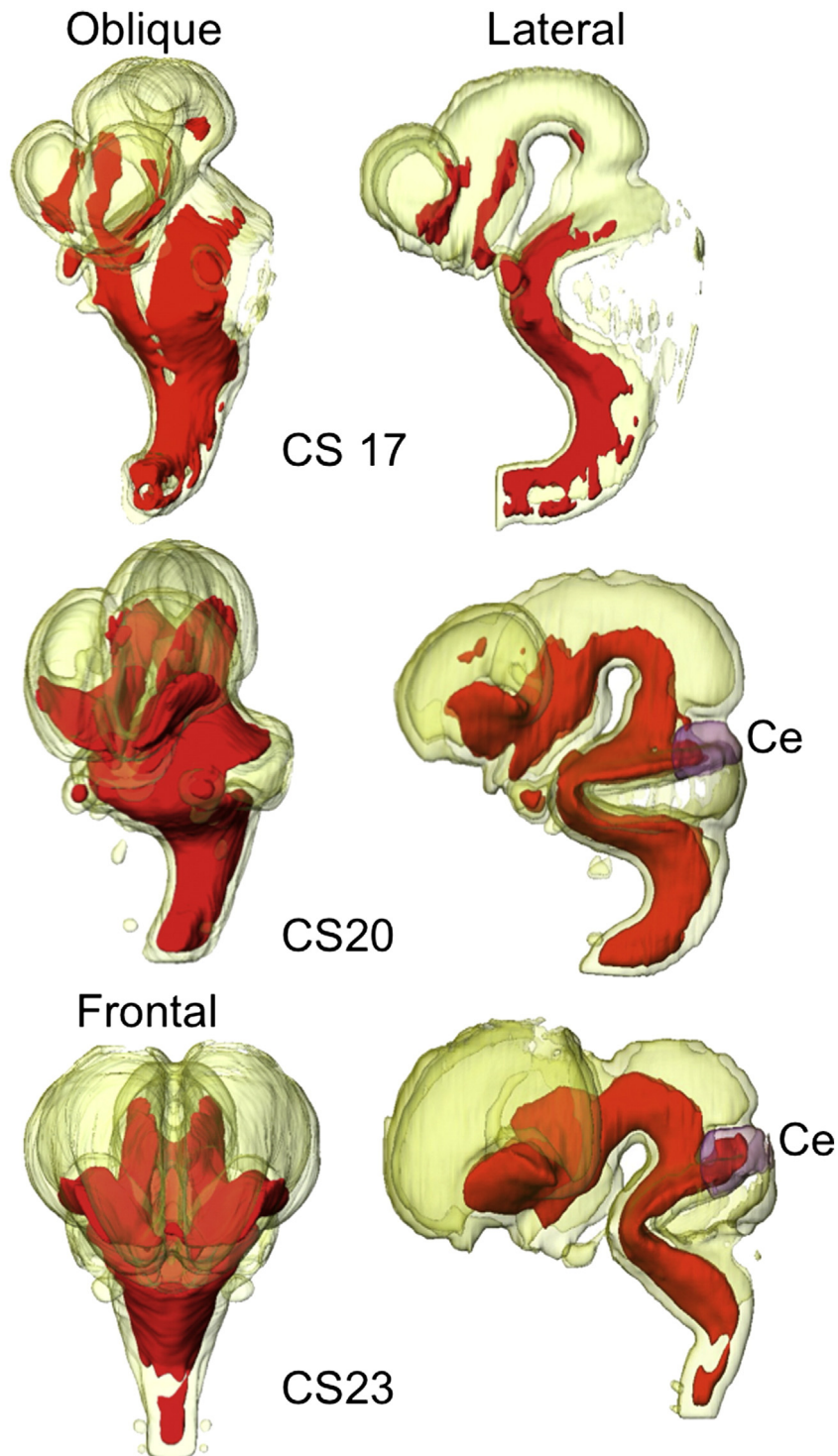


Fig. 6. The core region (COR) of the thickening brain tissue. The amount of tissue digitally subtracted to isolate the COR was determined using the dorso-lateral region of the telencephalon as a reference. The thickness subtracted was 0.28 mm for the CS17 brain, 0.4 mm for the CS20 brain, and 0.95 mm for the CS23 brain. Ce; the cerebellum.

brain throughout the fetal period and into postnatal development and adulthood (Dunn, 1921; Jenkins, 1921; Grenell and Scammon, 1943).

Embryonic development is characterized by dynamic and obvious changes in external appearance in accordance with internal organogenesis. To visualize the non-uniform regional thickness of the tissues of the embryonic brain during development we employed surface color mapping by thickness and extraction of the COR of the thickened brain tissue by the digital subtraction of layers of tissue. Brain tissue may grow in two directions. Eccentric growth occurs without

thickening of the brain tissue, while concentric growth results in brain tissue thickening. It is well known that flexion/extension occurs during the formation of the highly structured brain during the embryonic period. Surface mapping by thickness in the present study demonstrated the correlation between the dynamic structure (flexion/extension) and the direction of tissue growth (thickness). The tissue became thicker in the regions of flexion while it remained thin in the regions of extension. Surface mapping enabled us to visualize both surface morphology and internal thickness at the same time.

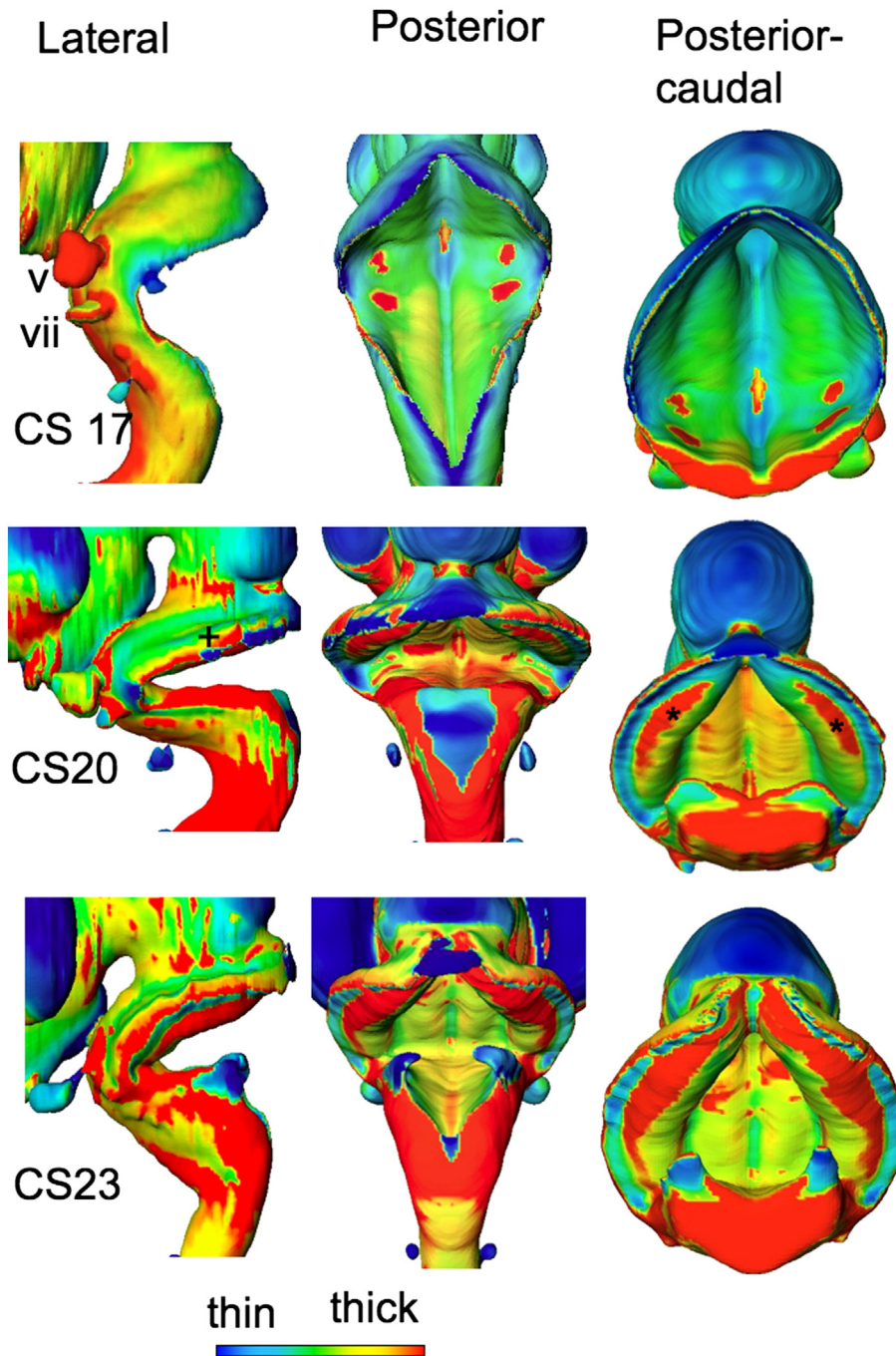


Fig. 7. Representative 3D images of the rhombencephalon with surface color mapping at CS17, 20, and 23. The roof of the rhombencephalon has been removed to reveal the inside tissues of the fourth ventricle, demonstrating the internal thickenings in the pons and medulla (brain stem) and cerebellar regions. *, Internal cerebellar swelling; +, rhombic lip. Closer view of the rhombencephalon is shown in Supplementary File 3 in Shiraishi et al. (submitted for publication).

The brain core was isolated by digital subtraction of layers of tissue from both the external and internal surfaces of the developing brain in the present study. This allowed us to visualize the COR of the thickening brain where concentric growth had occurred. The COR appeared to correspond to the growth of cerebral nuclei, cranial nerve nuclei, and pyramidal tracts when the anatomical positions of growth were compared with our own histological sections and an atlas of the embryonic brain (Gasser et al., 2014; Bayer and Altman, 2008) (Supplemental Fig. 1). The COR, as defined in the present study, may be valuable as an anatomical indicator of the development and differentiation of the nuclei and tracts in the brain. Histological study and/or brain imaging study using diffusion tensor image (DTI) techniques with fresh tissues of human

embryos are necessary to precisely reveal the development of the neural ganglia and tracts. Previous fetal brain imaging studies using diffusion tensor image have been performed in the second trimester (Huang et al., 2009). Although fresh tissues from human embryos are difficult to obtain, our methods may be suitable as a convenient alternative for studying the development and differentiation of the neural ganglia and tracts.

Recent morphological approaches to the study of human embryonic development have become multi-directional with the aid of high-resolution digital imaging. The development of the embryonic brain has been previously examined using histology-based methods. Multiple histological studies have been published, which describe the precise

local developmental anatomy. However, precise knowledge regarding the 3D volumes and shapes of structures within the developing human brain has been lacking. Gasser et al. (2014) has “rebirthed” a huge amount of histological data with an accessible and comprehensive database (<http://virtualhumanembryo.lsuhs.edu/>). The database consists of all levels of each sectional image shown with 3D reconstruction. Such embryo visualization programs provide valuable image data sets of human embryos for the education of students and obstetricians, as well as for the study of human development. The Human Developmental Studies Network (Kerwin et al., 2010, HuDSeN; <http://www.hudsen.org/>) is a unique database that aims to provide a forum for researchers in human developmental biology and related fields such as experimental developmental molecular and cellular biology. This network allows these researchers to establish links and exchange information. The integration and analysis of gene expression data from various stages of human development will enable a comparative analysis between human and mouse spatio-temporal gene expression data (HuDSeN human gene expression spatial database) in the future.

Clinically, sonography is convenient and low-risk compared to other imaging methods such as MRI and CT. Three-dimensional sonography, performed with high-frequency transvaginal transducers, has expanded the depth of inquiry and allowed 3D sonoembryology (Blaas and Eik-Nes, 2009; Pooh et al., 2011). These sophisticated sonographic imaging techniques allow the definition of in vivo anatomy including visualization of the embryonic blood circulation, cerebrospinal fluid flow, and other dynamic features that cannot be characterized in fixed specimens (Pooh, 2012a, 2012b; Pooh and Kurjak, 2011). In addition, 3D sonoembryology provides a basis for the assessment of anomalies as well as normal human development, and will contribute to more accurate prenatal diagnoses (Blaas, 2014).

Most MR imaging-based anatomical studies like ours have been limited by the use of expired embryos stored in formalin. Research using clinical data from sonography will be valuable as any artifacts, due to prolonged storage in formalin, would be eliminated (van Duijn et al., 2011). Our study does have the advantage of providing a convenient 3D reconstruction and quantitative morphometric assessment of the embryonic brain, especially regarding the dynamic changes that occur during brain development. The present study provides information complementary to information from histology, sonoembryology, and experimental biology. This greatly expands our understanding of brain growth and provides unique data for future studies.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2015.04.044>.

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Morphogenesis of Lateral Choroid Plexus During Human Embryonic Period

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ABSTRACT

The morphological and histological changes of the choroid plexus (CP) during Carnegie stage (CS) 18 and CS23 were presented, based on magnetic resonance imaging data and histological serial section of human embryos from the Kyoto Collection of Human Embryos. The primordium of the CP was initially detected as a small lump at CS19 that grew caudally, so that the CP became crescent shaped. It developed in all directions after CS21, as the dorsal and frontal growth also became prominent. The CP formed a number of undulating surfaces at CS20, irregular bulges at CS21, and then three large clusters with two deep fissures on the caudal surface at CS23. The mean volume of the CP was $0.282 \pm 0.141 \text{ mm}^3$ at CS19; it reached $16.8 \pm 8.77 \text{ mm}^3$ at CS23. Additionally, the histology was different depending on the regions of the CP at all stages after CS20. The epithelium and angioblasts in the center of the stroma were proliferated in the proximal region, whereas the epithelium was differentiated and lobulated in the distal region where the blood vascular system was organized. The histological differentiation was mapped on the CP reconstructed from histological serial sections. The data suggested the correlation between morphological information obtained from magnetic resonance data sets and distribution of the differentiation. With the help of morphological analysis and histological findings, we have been able to categorize each CP into specific stages. These findings will be useful in clinical evaluation of development during the embryonic period. *Anat Rec*, 296:692–700, 2013. © 2013 Wiley Periodicals, Inc.

Key words: lateral choroid plexus; human embryo; magnetic resonance imaging

Abbreviations used: CP = choroid plexus; CS = Carnegie stage; DV = diencephalic ventricle; JB = junctional base; LV = lateral ventricle; MR = magnetic resonance; 3D = three-dimensional.

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TABLE 1. Stage of differentiation of human lateral ventricular choroid plexus

	Stage I	Stage II
Time of development	Seventh week of gestation	Nineth week of gestation
Epithelium	Pseudostratified tall; Predominantly central nuclei	Low columnar; apical nuclei
Glycogen	Absent?	Abundant
Villi	Absent; lobules present	Sparse primary villi
Tubules	May be present	Several
Stroma	Loose mesenchyme	Extremely loose mesenchyme, small amount of connective tissue fibers
Stromal blood vessels	Islets of nucleated blood cells surrounded by ill-defined vascular walls, blood islets located subepithelially	Definite vascular walls, capillaries located subepithelially, large blood vessels in central interstitium
Size of plexus in relation to ventricle	Minute	Extremely large

The human brain is arguably one of the most complicated organs in living systems. It develops after the dynamic and gross changes during the embryonic period (Moore et al., 2008). Originating from a simple neural tube, this elaborate structure is formed by a series of differentiation processes (Bayer and Altman, 2002; Huang et al., 2009). Neural tube closure in the human embryo initiates at multiple sites in Carnegie stage (CS)10 (22 days after fertilization) and completes at CS12 (26 days after fertilization) (Nakatsu et al., 2000). The cerebral vesicle, formed at the cranial end of the neural tube, differentiates and forms the prosencephalic ventricle, the mesencephalic ventricle, and the rhombencephalic ventricle at CS13. The prosencephalic ventricle is divided into diencephalic ventricle (DV) and telencephalic ventricle at CS14, which become the lateral ventricle (LV).

The choroid plexus (CP) is a special organ that produces cerebrospinal fluid. It has a unique structure, differentiated from ependymal cells, and is responsible for an important biological barrier system, forming an interface between the blood and the cerebrospinal fluid. The CP is distributed in four parts, one in each of the LVs, one in the DV, and one in the rhombencephalic ventricle. Precise studies about the development of the CP have been published for a number of species: rabbit (particularly vascularization of the CP) (Strong, 1956, 1964a,b), sheep (Jacobsen et al., 1983), mouse (Knudsen, 1964; Sturrock, 1979), and rat (Wislocki and Ladman, 1958; Cancilla et al., 1966). In humans, primordium of the CP forms on the roof of the telencephalon medium (choroid plate) as a fold at CS17 and CS18 (Müller and O'Rahilly, 1990; O'Rahilly and Müller, 1990). The primordium develops to CP of the bilateral LVs and DV accompanies with the differentiation of those ventricles. Ariens Kappers (1958) has categorized the development of the lateral ventricular CP between 6 weeks and 5.5 months of gestation into three histogenetic phases, according to the histological findings such as epithelium type and the presence of cytoplasmic glycogen. Netsky and Shuangshoti, (1975) also observed the CP of the fetus and the neonate at 1 month after birth, then extended this to four stages (Table 1). Though their study was excellent, the information about embryonic period was insufficient. According to their categorization, only the first and a part of the second stage may include the period during CS19 and CS23 although the cerebral vesicles develop

dramatically in those periods (O'Rahilly and Müller, 2006).

In this study, the morphogenesis of the CP of the LV region between CS19 and CS23 were precisely observed applying two approaches—three-dimensional (3D) reconstruction using magnetic resonance (MR) imaging data and histological observation. The data provided demonstrate the dramatic growth of CP during the embryonic period in each CS.

MATERIALS AND METHODS

Human Embryo Specimens

Approximately, 44,000 human embryos comprising the Kyoto Collection are collected and stored at the Congenital Anomaly Research Center of Kyoto University (Nishimura et al., 1968; Shiota, 1991; Yamada et al., 2004). In most cases, pregnancy was terminated during the first trimester for socioeconomic reasons, under the Maternity Protection Law of Japan. Some of the specimens (~20%) were undamaged, well-preserved embryos. When the aborted embryos were brought to the laboratory, they were measured, examined, and staged using the criteria provided by O'Rahilly and Müller (1987). Approximately, 500 well-preserved human embryos, diagnosed as externally normal from CS13 to CS23, were subjected to histological serial sections. Another set of 1,200 well-preserved human embryos were selected for MR microscopic imaging. The conditions used to acquire the serial sections and the MR images of the embryos are described elsewhere (Nishimura, 1975; Matsuda et al., 2003; Yamada et al., 2006; Shiota et al., 2007).

For this study, 49 samples distributed between CS19 and CS23, consisting of 10 samples for each stage (except CS22 for which there were nine samples) were selected from the 1,200 MR image data sets, based on the criteria described previously (Nakashima et al., 2011) for 3D reconstruction and morphometric analysis. Forty-one samples distributed between CS18 and CS23, consisting of seven samples for CS18 and CS22, six samples for CS19, five samples for CS20 and CS23, and 11 samples for CS21, were selected for histological analysis, based on the following criteria: no obvious damage or significant anomaly is present in the cerebral vesicle, ventricle, abdominal organs, and external appearance.

3D Reconstruction and Morphometric Analysis

3D MR image data sets for each embryo were initially obtained as $256 \times 256 \times 512$ voxel data (Matsuda et al., 2007). Sequential 2D images were resectioned digitally by using ImageJ64™ (ver. 1.44, National Institutes of Health, Bethesda, Maryland) and saved as Analyze file formats (.hdr, .img). The LV and the CP were segmented for 3D reconstruction using FSL View of FMRIB Software Library™ (ver. 4.1.9, Analysis Group, FMRIB, Oxford, United Kingdom). Histological serial sections for 3D reconstruction were digitized using a film scanner (DiIMAGE Scan Multi PRO AF-5000, Konica Minolta, Tokyo, Japan). 3D objects of CP were computationally reconstructed and morphometrically observed in software Amira™ (ver. 5.4.0, Visage Imaging, Berlin, Germany). Volume of the CP was calculated using the software OsiriX™ (ver. 4.0, Pixmeo SARL, Geneva, Switzerland).

Histological Observations

Histological serial sections stained with Hematoxylin and Eosin were observed microscopically by two of the authors (Naoki Shiraishi and Tetsuya Takakuwa). The histological findings of the CP were evaluated according to the previous study (Shuangshoti and Netsky, 1966) (Table 1), that is 1) type of epithelium and the presence of glycogen in the cytoplasm, 2) position and mitosis of the nucleus in the epithelium, 3) connectivity and lobulation of the stroma, 4) the presence of angioblasts, and 5) the presence of the capillaries. The degree of differentiation was categorized into three stages using the histological findings, that is Dif: histologically differentiated (simple columnar epithelium, apical nuclei, and vacuoles in the cytoplasm), Undif: histologically undifferentiated (pseudostratified epithelium, frequent mitosis, and many angioblasts in the stroma), and Mix (the histological findings of both Dif and Undif mixed in various combinations).

RESULTS

Morphogenesis of the Reconstructed CP

The CP was arising from areas close to the interventricular foramen and close to the medial eminence depression by the amygdala in CS18. The primordium of CP was detected by histology, as described later, but not by 3D reconstruction (Fig. 1). The primordium formed a small lump at CS19, and then developed in the LV and DV. The left and the right CP developed as mirror images, implying no noticeable morphological differences between the two. The growth of the CP followed that of the LV. The LV formed a C-shape between CS18 and CS23. The inferior horn grew like the “horn of a ram” (O’Rahilly et al., 1987; Drews, 1995), constructing an arch directed backward. A similar development was also observed for the growth toward the caudal direction between CS18 and CS21 (Figs. 1, 2). The CP developed laterally and caudally at CS20. It was thin and slightly curved (crescent shaped). It grew caudally toward the inferior horn of the LV and developed in all directions after CS21. Growth in the dorsal direction was prominent after CS22. Further, the CP rapidly grew frontally and

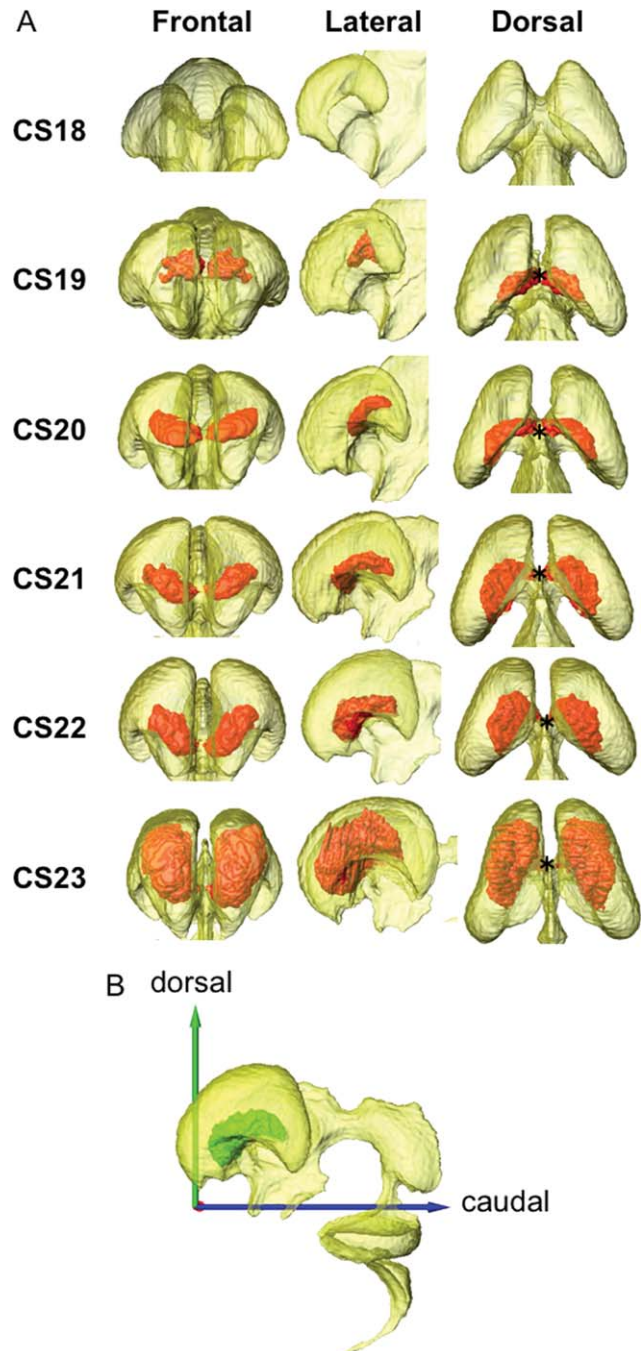


Fig. 1. Representative 3D image of the CP (red) within the LV (yellow). (A) Frontal, lateral, and dorsal view between CS18 and CS23 are shown. Asterisk (*) in the dorsal view indicates the JB. (B) Orientation used in this article: The red, green, and blue arrows indicate lateral, dorsal, and caudal views, respectively.

filled one-fourth of the LV at CS23 (Supporting Information video S1).

The surface of the CP changed according to the development and differentiation. The CP formed a number of undulating surfaces at CS20, and then formed irregular bulges on the caudal–dorsal surface at CS21. The bulges became more complicated and deep in CS22 (than in

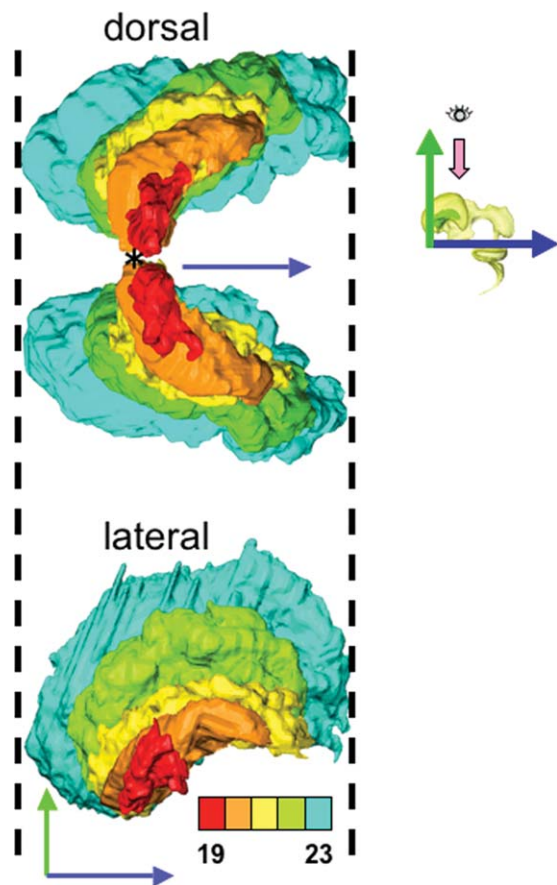


Fig. 2. Direction of growth of the CP between CS19 and CS23. For comparison, CP between CS19 and CS23 was merged at the JB, with the same magnification. Dorsal and lateral views are shown. Asterisk (*) in the dorsal view indicates the JB. The viewpoint was indicated in the illustration of the ventricles.

CS21); they were more on the caudal surface, whereas a few were on the frontal surface. Several large clusters on the caudal surface were observed in place of the bulges of the surface at CS23 (Fig. 3A). Three large clusters with two deep fissures were observed in 7 out of 10 cases (Fig. 3B). These clusters were distributed in a medial to lateral order.

The CP facing in DV was too small to be recognized in the MR imaging data at all stages analyzed in this study.

Volume of CP and LV

The mean volume of the CP was $0.282 \pm 0.141 \text{ mm}^3$ (mean $\pm$ SD) at CS19. It exponentially increased until CS23 when it reached $16.8 \pm 8.77 \text{ mm}^3$ (Fig. 4). The CP increased in volume by approximately 60-fold from CS19 to CS23. The mean volume of the LV was $6.10 \pm 1.43 \text{ mm}^3$ at CS19. It exponentially increased until CS23 when it reached $74.3 \pm 28.0 \text{ mm}^3$. The volume ratio of CP to LV increased from $4.84 \pm 2.52\%$ to $21.5 \pm 4.13\%$ between CS19 and CS23. That is, the CP was increasing in volume relatively faster than the LV. The intrastage variation of the CP to LV ratio was limited. There was

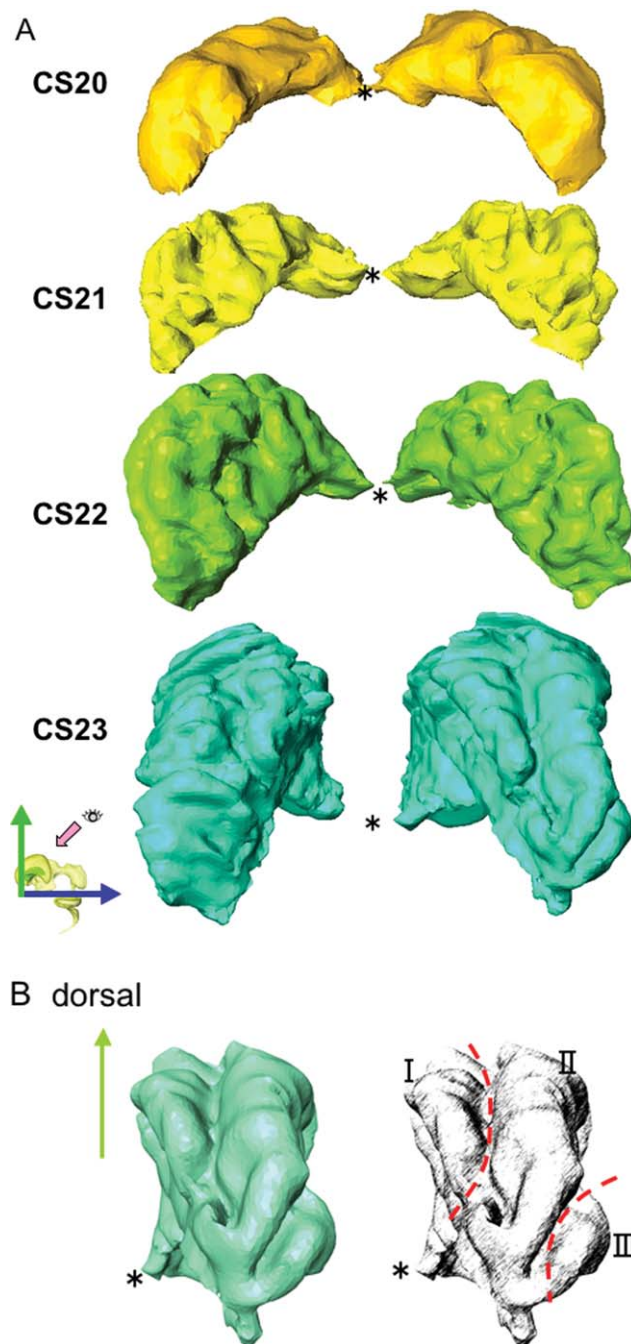


Fig. 3. Representative gross 3D images of the CP. (A) The feature of the dorsal surface between CS20 and CS23. The viewpoint was indicated in the illustration of the ventricles. (B) Dorsocaudal view of right lateral CP in CS23. Three large clusters (I, II, and III) with two deep fissures (red dot) are indicated in schema. The similarity between these clusters and the microvascular architecture in the fetal period has been discussed in Discussion section. Asterisk (*) in the dorsal view indicates the JB.

no significant difference in volume between the right and the left CP (data not shown). The present data were consistent with the morphological observations described above.

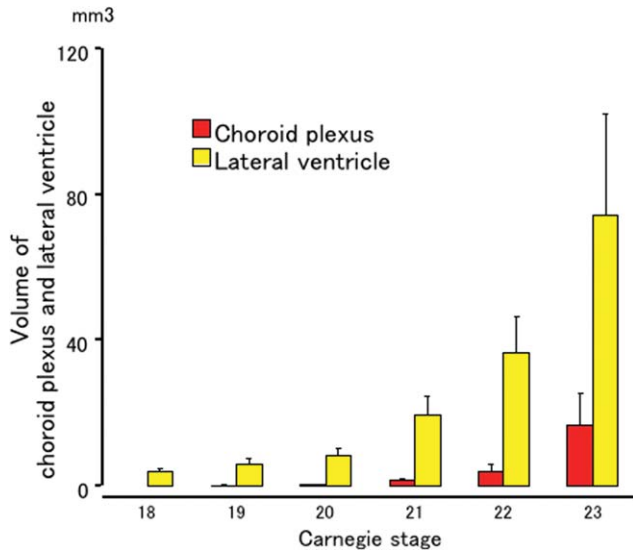


Fig. 4. Calculated volumes of LV and CP between CS18 and CS23. The volume was calculated as described in Materials and Methods section. Volume at each CS is shown as mean $\pm$ standard deviation (mm^3).

Histology of the CP

In all cases, the primordium of the CP of the LV region appeared at CS18 (Fig. 5A-a and Table 2). The border, as evident by histology, was defined as “junctional base (JB)” and used as the anatomical reference in this study. The CP was covered with pseudostratified tall epithelium. The epithelium was thinner than the neuroepithelium (Fig. 5A-b,c). The nuclei of the epithelium were large and centrally located in almost all areas. The frequency of nuclear mitosis in the epithelial cells was comparable with that of the neuroepithelium. The stroma was loose and many nucleated red-stained cells were found at the subepithelial regions; these cells were defined as angioblasts (Ellen, 1922).

The primordium became club shaped in four out of the six cases (66.7%) at CS19. The epithelium was not uniform in thickness, that is the basal membrane (boundary between epithelium and stroma) was irregular (Fig. 5B-a). The pseudostratified epithelium intruded into the stroma in spots. In those spots, the epithelium became thinner as the rows of pseudostratified cells reduced in number (Fig. 5B-b). A mitotic figure was observed at the luminal side of the epithelium, at the same degree as in the neuroepithelium. Numerous angioblasts were observed in the central region of the stroma, and endothelial cells were occasionally observed around them (Fig. 5B-c).

The fist-shaped CP protruded toward LV in all cases at CS20 (Fig. 5C-a). The irregularity of epithelium was conspicuous in four out of the five cases (80.0%). Interlobular clefts were observed in three out of the five cases (60.0%) (Fig. 5C-b). The CP was mostly covered with pseudostratified tall cells, but a few tall columnar epitheliums with apically elevated nuclei were present at the distant region from the JB. Mitotic nuclei were present at the apical side of the epithelial cells as much as at the neuroepithelium. Numerous angioblasts were located subepithelially, indicating the formation of the capillary and vein (Fig. 5C-c).

The lobulation of the CP proceeded in CS21 (Fig. 5D-a); some tubules were observed in 8 out of the 11 cases

(72.7%) and primary villi appeared in 2 out of the 11 cases (18.2%). The tall columnar epithelium covered an apparently much wider area at CS21 than at CS20. In the epithelium, the nuclei were distributed at the apical border in 8 out of the 11 cases (72.7%). The pseudostratified epithelium had a few lobulations and frequent mitosis, and the stroma had many angioblasts in the region close to the JB (Fig. 5D-b), whereas the tall columnar epithelium in distal region had almost apically elevated nuclei, few mitosis, many lobulations, and the stroma had a few angioblasts and frequent capillary walls (Fig. 5D-c). These findings suggest that the proximal region of CP was undifferentiated and proliferated, whereas the distal region was differentiated at CS21.

Pseudostratified epithelium and numerous angioblasts were still observed in the proximal region at CS22, whereas the other epithelium formed a single layer of cylindrical, ciliated cells with apically lined oval nuclei. Vacuoles were observed in the basal side of the cytoplasm within the epithelial cells in four out of the seven cases (57.1%). These epithelial cells may have cytoplasmic glycogen (Shuang-shoti and Netsky, 1966). The CP lobulated in a complex manner and many tubules and primary villi were observed in six out of the seven cases (85.7%). In the distal region, the stroma was extremely loose, angioblasts were few, capillaries were formed subepithelially, and veins were scattered in the central interstitium (data not shown).

The CP expanded with almost no lobulation in the distal region in CS23 (Fig. 5E-a). Almost all the CPs were covered with a single layer of low columnar epithelium, except the region in the stalk or the tips of the interlobular clefts (Fig. 5E-b,c). Nuclei were located apically and mitosis was rarely observed in the single layer of low columnar epithelium. The stroma was extremely loose and angioblasts were rarely seen.

Histological Differentiation Map on Reconstructed CP

Histological observation suggested that the histological feature in the proximal region was different from that in the distal regions of the CP after CS20. In this context, the histological differentiation was then mapped on the CP reconstructed from histological serial sections.

The 3D reconstructed image from histological serial sections at CS21 revealed features similar to that obtained from MR data sets (Fig. 6, Supporting Information videos S2,S3); it revealed a crescent shape with thickness in the dorsoventral direction, and an irregular bulge formed at the caudal–dorsal region.

The histological differentiation map revealed the Undif located at the frontal–ventral region, close to the JB (Fig. 6); the Dif located at the frontal–dorsal region, which is distant from the JB; and the Mix located at the caudal region. The ratio of the Undif and Dif to the total CP in volume was 27.0% and 28.7%, respectively. The junction between the CP and the medial wall of LV become broad, and stretched from the JB (asterisk, Fig. 6) to the caudal regions.

A 3D image at CS23 was also reconstructed from histological serial sections (Supporting Information video S4). Undulation and large clusters were observed in the caudal surface of the histological reconstructed CP at CS23; these were also detected on the 3D image from MR imaging data (Fig. 3, Supporting Information video

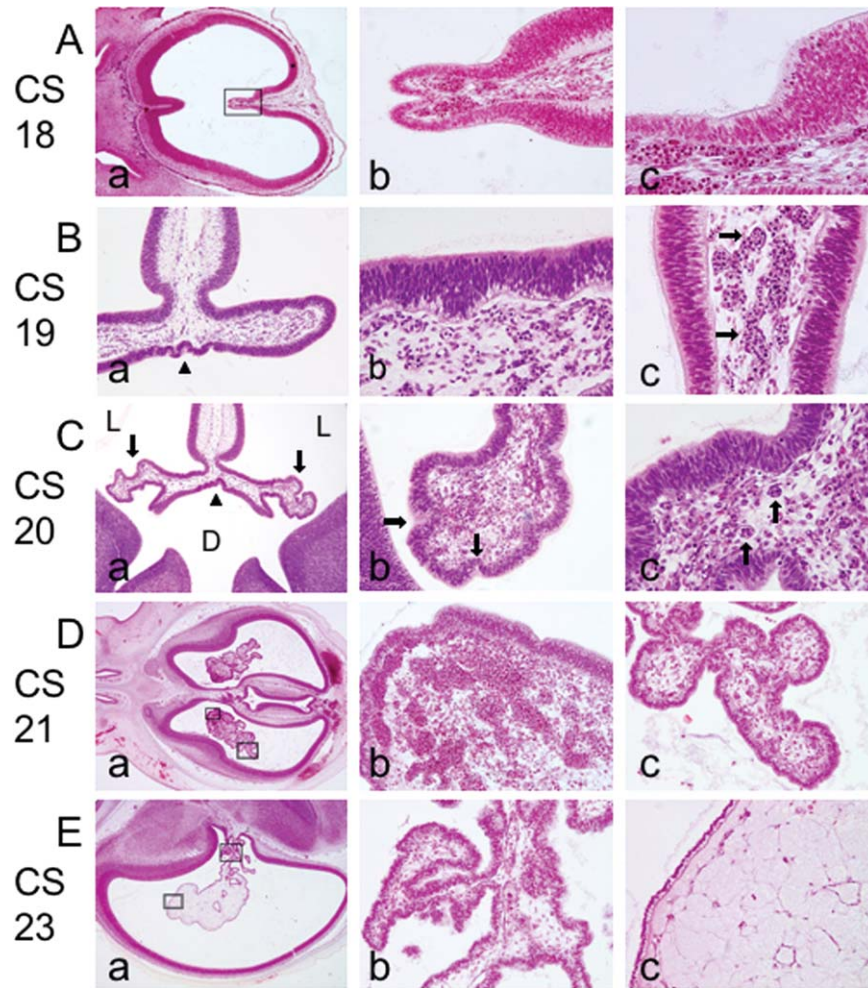


Fig. 5. Histology of the CP in LV stained with Hematoxylin and Eosin. Each magnification is indicated in parentheses. **(A)** Coronal section of the CP at CS18 (No. 1633). (a) The primordium of CP form on the roof of the telencephalon medium (choroid plate) (squared) ($\times 40$). (b) ($\times 200$). (c) The junction between CP and ventricular wall. Thickness of the pseudostratified epithelium differs between them. The junction was defined as JB in this study for the reference point ($\times 400$). **(B)** Coronal section of the CP at CS19 (No. 4424). (a) The CP forming a "Club" shape. ($\times 100$) Arrowhead ($\blacktriangle$) indicates the paraphysis. (b) The epithelium showing nonuniform thickening ($\times 400$). (c) Angioblasts with endothelial cells (arrows) ($\times 400$). **(C)** Coronal section of the CP at CS20 (No. 1770). (a) The CP forming "fist-shape" (arrow). Note that the roof of the telencephalon grows and divides between LVs (L) and DVs (D). The epithelium facing the DV is tall, indicating ventricular wall

(neuroepithelium) but not CP ($\times 40$). Arrowhead ($\blacktriangle$) indicates the paraphysis. (b) The epithelium in the CP of LV, showing interlobular clefts (arrows) ($\times 200$). (c) Angioblasts with vascular wall in the stroma (arrows) ($\times 400$). **(D)** Coronal section of the CP at CS21 (No. 2314). The specimen belongs to the late half of CS21. (a) More complex lobulation of the CP and appearance of villous structure (primary villi) ($\times 20$). (b) The region close to the junction with ventricle wall (square in (D-a)) ($\times 200$). (c) The region distant from the junction (square of doublet in (D-a)). Villous structure was observed ($\times 200$). **(E)** Sagittal section of the CP at CS23 (No. 4381). (a) Gross view ($\times 20$) (b) The region close to the JB (square in (E-a)). Tubular and villous structure was observed ($\times 200$). (c) The region distant from JB (square of doublet in (E-a)) ($\times 200$).

S1). Dif, located at the distal region of CP, occupied approximately 81.2% of the CP by volume. Mix occupied the remaining proximal region. Undif appeared rudimentary at the proximal region of CP. The junction between the CP and the medial wall of the LV continued from the JB (asterisk, Fig. 6) to the caudal regions, which is similar to that at CS21.

DISCUSSION

The morphogenesis of CP in the human embryonic period have not been fully described so far. Shuangshoti

and Netsky (1966) described histological findings in 18 cases of fetus including several cases of embryo with crown-rump length of 13–30 mm. O'Rahilly and Müller (1990) described the morphogenesis of cerebral ventricles between CS11 and CS23. The histological feature of CP was described in that study as a specific region of the cerebral ventricle between CS18 and CS23. The emergence of the primordium of CP was precisely described. However, morphological and histological data corresponding to each CS remain questionable. In this study, the morphogenesis of the lateral ventricular CP between CS18 and CS23 using 3D reconstruction and precise

TABLE 2. Acquisition of histological feature of the CP from CS 18–23

Structure	State	Change	CS					
			18	19	20	21	22	23
Epithelium	Type	Only pseudostratified	7 (100)*	6 (100)	4 (80.0)	5 (45.5)	0	0
		Pseudostratified+ low columnar	0	0	1 (20.0)	6 (54.5)	6 (85.7)	1 (20.0)
		Completely low columnar but stalk	0	0	0	0	1 (14.3)	4 (80.0)
Nuclei	Glycogen	No presence	7 (100)	6 (100)	5 (100)	10 (90.9)	3 (42.9)	0
		Presence	0	0	0	1 (9.09)	4 (57.1)	5 (100)
	Position	Predominantly central	7 (100)	6 (100)	4 (80.0)	3 (27.3)	0	0
		Central and apical mixed	0	0	1 (20.0)	8 (72.7)	5 (71.4)	1 (20.0)
		Predominantly apical	0	0	0	0	2 (28.6)	4 (80.0)
	Mitosis	Presence	7 (100)	6 (100)	5 (100)	9 (81.8)	6 (85.7)	0
Stroma	Connectivity	No presence	0	0	0	2 (18.2)	1 (14.3)	5 (100)
		Loose	7 (100)	6 (100)	5 (100)	11 (100)	1 (14.3)	0
	Lobulation	Extremely loose	0	0	0	0	6 (85.7)	5 (100)
		No lobulation	7 (100)	6 (100)	2 (40.0)	1 (9.09)	0	0
		Lobulation	0	0	3 (60.0)	2 (18.2)	0	0
		Lobulation+tubules	0	0	0	6 (54.5)	1 (14.3)	0
		Lobulation+ tubules+villi	0	0	0	2 (18.2)	6 (85.7)	5 (100)
Blood vessels	Angioblast	Numerous	7 (100)	6 (100)	5 (100)	6 (54.5)	1 (14.3)	0
		A few	0	0	0	5 (45.5)	6 (85.7)	5 (100)
	Capillaries located subepithelially	No presence	7 (100)	6 (100)	5 (100)	1 (9.09)	1 (14.3)	0
		Presence	0	0	0	10 (90.9)	6 (85.7)	5 (100)
			7	6	5	11	7	5

*Percentage is given in parentheses.

histological observation are described (Fig. 7). The 3D reconstruction model of the CP revealed unique and discernible morphology at each CS.

As previously described, the primordium of CP was observed only histologically at CS18. The CP mainly grew caudally along the inferior horn of the LV between CS18 and CS21; it grew in all directions after that stage. The direction of growth does not seem to be restricted to the shape of the ventricle because of leaving enough room within it during the embryonic period. The CP filled approximately one-fourth of the LV at CS23. The speed of growth is maintained until the CP filled approximately three-fourths of the LV at 50 mm crown-rump length (around 11 weeks of gestation) (Shuangshoti and Netsky, 1966).

The surface of the CP was also unique at each stage. The CP had a number of undulating surfaces at CS20, irregular bulges at the caudal–dorsal surface at CS21, and numerous deep bulges on the caudal surface at CS22. They formed a large cluster at CS23. The distribution of the clusters observed in 3D reconstruction at CS23 was similar to that of microvascular architecture found in human fetuses at 20 weeks of gestation as previously reported (Zagorska-Swiezy et al., 2008). In that study, the CP was divided into five clusters based on the microvascular pattern and density—the anterior part, the glomus, the posterior part, the villous fringe, and the free margin. Three large clusters observed at the caudal surface in reconstructed CP at CS23 may correspond to the villous fringe, glomus, and posterior part because of their location and morphological features. Analysis of the development of microvascular architecture during embryonic period will be necessary to decide whether the clusters observed in this study are identical to those structures or not.

Each histological finding of CP was similar to those described in the previous studies (Netsky and Shuangshoti, 1975; Müller and O'Rahilly, 1990). However, the histology may be different depending on the regions of the CP. The epithelium and angioblasts in the center of the stroma were proliferated in the proximal region, whereas the epithelium was differentiated and lobulated, and had abundant cytoplasmic glycogen in the distal region where the blood vascular system was organized, that is capillaries were formed subepithelially and small veins were scattered in the central interstitium. A mitotic figure was rarely found. A similar tendency was observed at all stages after CS20. These data suggest that the CP proliferated mainly at the proximal regions and the differentiating components may have been pushed out to the peripheral region one after another.

3D reconstruction from histological serial images was used in this study because that approach has a definite advantage. In this approach, histological information can be mapped on the 3D image. 3D reconstruction from MR data sets is accurate in morphology (Nishimura, 1975; Matsuda et al., 2003; Yamada et al., 2006; Shiota et al., 2007). Comparison of the construction with the images obtained from MR reconstruction suggests that the histologically undifferentiated region may correspond to the area with smooth surface (mainly located at the frontal–medial part of CP) in MR reconstruction, whereas the histologically differentiated and mixed regions may correspond to the area with a lot of bulges, undulation, and/or clusters (mainly located caudal–lateral part of CP) in MR reconstruction. It is notable that the reconstructed CP from the two different approaches with different specimens was similar to each other. As

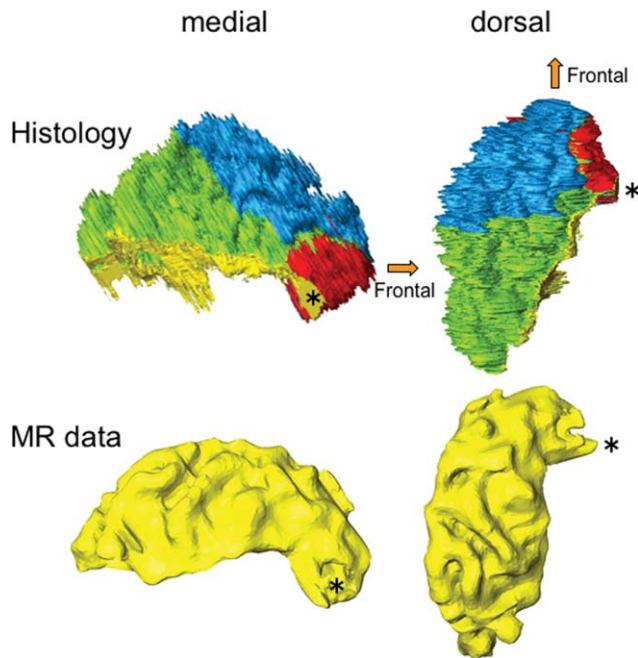


Fig. 6. 3D histological reconstitution map of left CP at CS21. 3D image of CP at CS21 was reconstructed using histological serial sections (No. 2314), and mapped the histological differentiation; red: undifferentiated, blue: differentiated, green: mixed. The definition of the differentiation was described in Materials and Methods section. Yellow: the junction between CP and ventricle wall. The reconstructed CP from MR sets were shown for comparison. Asterisk (*) indicates the JB.

the comparison in this study was limited in number and stages, further analysis may be necessary.

The JB defined in this study was valuable for anatomical reference. The JB arose as the border between the telencephalic ventricle wall and the primordium of the CP at LV region at CS18. The junction was very limited until CS20. With the development of the CP of LV regions, the junction between the CP and the wall of the LV become broad, elongating from the JB in the caudal direction after CS21. That junction may be necessary for abundant blood supply to the CP (Millen and Woollam, 1953).

The CP of the DV and paraphysis may arise around the JB because the CP of LV and DV are continuous in the mammalian brain (Netsky and Shuangshoti, 1975; Dziegielewska et al., 2001). O'Rahilly and Müller (1990) observed it at CS18 for the first time. In this study, the primordium faces both LV and DV at CS 20. Paraphysis was detected at least after CS19 at the roof of the DV and the epithelium became thin and irregular around that, whereas the stroma remained undifferentiated. This study did not determine whether structures around the paraphysis were CP of the DV or part of the paraphysis.

The CP is a special organ with a unique morphology. It produces cerebrospinal fluid. Neoplasia (papilloma) of the CP causes serious diseases like hydrocephalus (Eisenberg et al., 1974). This study reveals that the CP has a characteristic differentiation and morphogenesis phase between CS18 and CS23 (Fig. 7). With the help of morphological analysis and histological findings, we have been able to categorize each CP into specific stages.

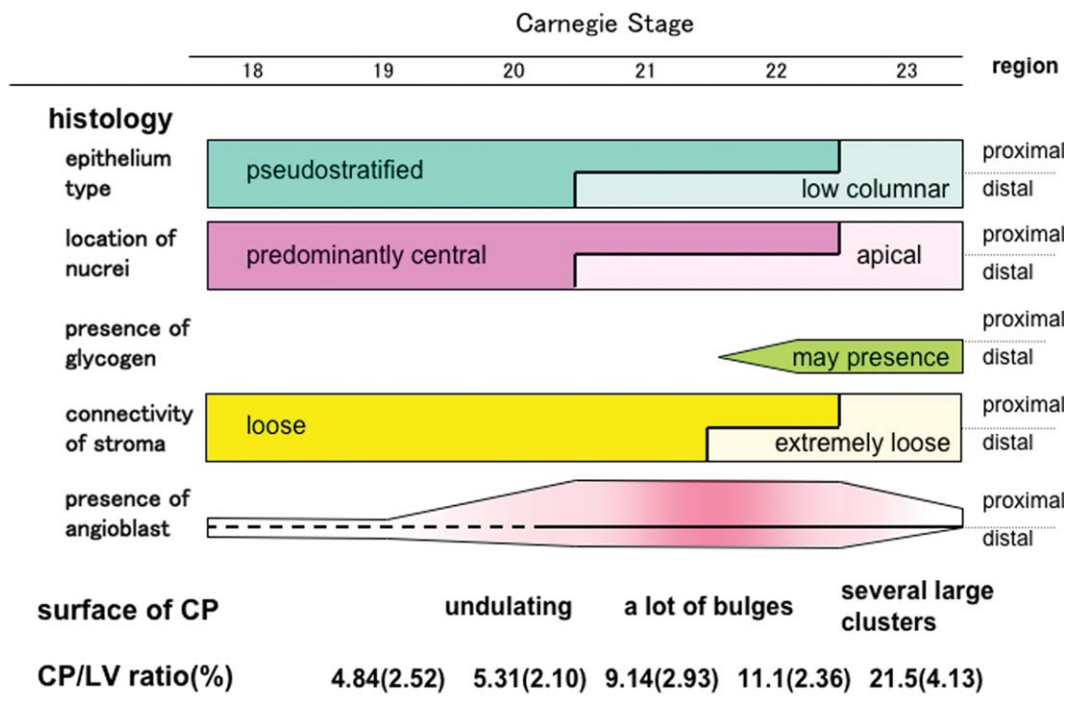


Fig. 7. Summary of histological findings of CP between CS18 and CS23. CP/LV ratio; ratio of volume of CP to volume of LV. Standard deviation (SD) of them is in parentheses. Note that intrastage variation of the ratio was limited except at CS23.

These findings will be useful in clinical evaluation of development during the embryonic period.

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LETTER TO THE EDITOR

Three-dimensional models once again: For research and teaching of early human development

Dear Editor,

Many people – some scientists, others not – are interested in early human development because it is closely related to the basic and important question: What are we and where have we come from?

Classically, histological serial sections were prepared for microscopic two-dimensional (2D) analysis as well as for understanding the three-dimensional (3D) development of external and internal structures (O’Rahilly and Müller 1987). 3D plaster or wax models, constructed by technical specialists since the late 19th century, have been indispensable in research as well as teaching (Morgan 2004). The histological models of Ziegler, Blechshmidt, and Heard are famous worldwide. The construction process of these models used to be so complicated that a team of scientists, technicians, artists and photographers spent several months to years to complete one model. Then, application of newer modeling techniques produced much more detailed models and contributed to vivid understanding of early human development.

With the advent of magnetic resonance imaging (MRI), the imaging of embryos has become highly effective, providing a resolution of 40 µm/pixel or more with long scan times. MRI is a non-invasive and non-destructive method that has many possibilities, not only for precise morphological observations but also for morphometric evaluations of internal structure (Yamada et al. 2006). A beautiful image on the 2D screen can be easily drawn from MRI digital data using computer graphics. However, we think it still fails to illuminate dynamic morphogenesis, particularly spatial changes and anatomical relationships.

A 3D printer is a tool for making 3D solid objects from digital data. Stereolithography was developed in 1986 and attracted much attention (Jacobs 1992). It did not become very popular because it involved very expensive equipment and many other incidental

facilities. In recent years, fused deposition modeling (FDM) method has been developed. It has enabled creation of inexpensive 3D models in engineering, medical and dental fields as well as the academic area (Dimitrov et al. 2006).

We constructed a series of 3D precise models of the central nervous system and cerebral ventricle of human embryo from MR data. We created the models according to the manufacturer’s operation manual (BFB-3000, Bristol, UK). These were 3D models of the cerebral ventricle between Carnegie stage (CS) 13 and 23. The printed 3D models allowed us to directly visualize the 3D appearance, touch and feel. Comparison of successive specimens enabled us to recognize the dynamic still precise morphological change during development, and to understand the spatial relationship and dynamism of morphogenesis more intuitively, as expected (Fig. 1).

The use of 3D printed models is expected to compensate the faults and limits of computed graphical pictures reconstructed on 2D display. The models are also expected to act as attractive and intelligible tools for teaching. We plan to teach the whole process from MRI data to visible embryonic models.

The plaster model played a substantial role at the dawn of research and teaching of human embryos. We expect that the 3D printed model will again be available and play an important role in the near future.

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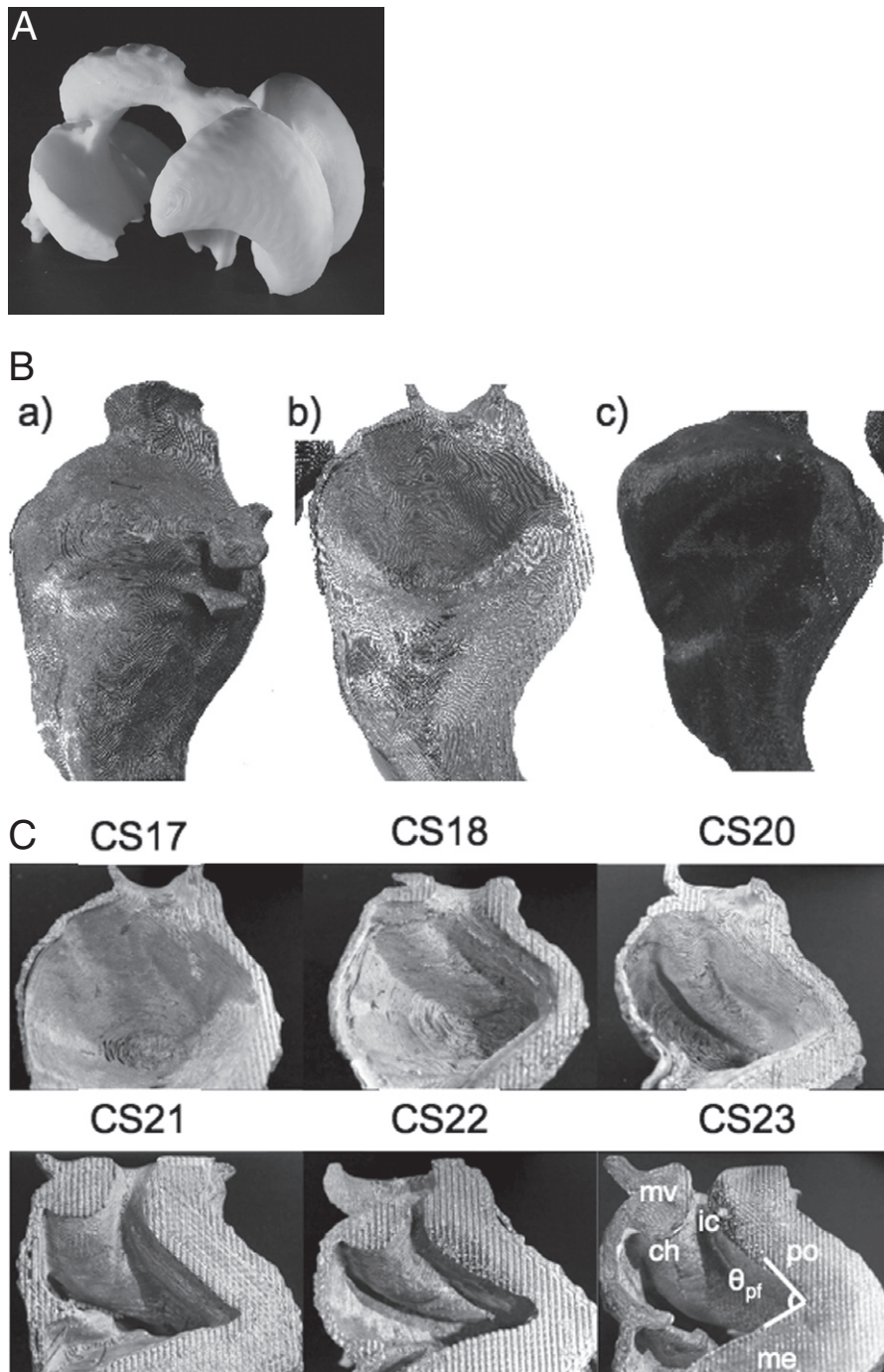


Fig. 1 (A) 3D model of cerebral ventricles at Carnegie Stage (CS) 23 printed by Fused Deposition Modeling method. (B) 3D models of the rhombencephalon (a,b) and rhombencephalic ventricle (c) at CS 17. (a) Right lateral surface view of the rhombencephalon. (b) Internal surface view of the left half of the rhombencephalon. (c) Right lateral view of the rhombencephalic ventricle with the same scale. Development of the rhombencephalon will be better understood by comparing the 3D model of the rhombencephalic ventricle with the rhombencephalon. (C) Internal views of the left halves of the 3D models of the rhombencephalon between CS17 and 23. Comparison of successive specimens enabled recognition of the dynamic still precise morphological changes about the cerebellum, brainstem and roof of the rhombencephalic ventricle and their effects on the rhombencephalic ventricle and pontine flexure. The cerebellar hemisphere arose from the rhombic lip and grew in thickness between CS17 and 23. The pons and medulla grew in thickness rapidly, thereby developing the pontine flexure. Mesencephalon and rhombencephalon were separated by the isthmus canal, which was narrowed by development of medullary velum and pons. ch, cerebellar hemisphere; ic, isthmus canal; me, medulla; mv, medullary velum; po, pons; θ_{pf} , pontine flexure.

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Data Article

Three-dimensional morphology of the human embryonic brain



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ABSTRACT

The morphogenesis of the cerebral vesicles and ventricles was visualized in 3D movies using images derived from human embryo specimens between Carnegie stage 13 and 23 from the Kyoto Collection. These images were acquired with a magnetic resonance microscope equipped with a 2.35-T superconducting magnet. Three-dimensional images using the same scale demonstrated brain development and growth effectively. The non-uniform thickness of the brain tissue, which may indicate brain differentiation, was visualized with thickness-based surface color mapping. A closer view was obtained of the unique and complicated differentiation of the rhombencephalon, especially with regard to the internal view and thickening of the brain tissue. The present data contribute to a better understanding of brain and cerebral ventricle development.

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Specifications table

Subject area	Biology
More specific subject area	Developmental anatomy

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Type of data	Three-dimensional image
How data was acquired	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.
Data format	Movie (.mov, .mp4)
Experimental factors	Well-preserved human embryos obtained mainly by induced abortion was fixed with formalin and selected for MR microscopic imaging.
Experimental features	Three-dimensional morphogenesis of the human embryonic brain between Carnegie stages 13 and 23 was visualized from MRI.
Data source location	Kyoto and Tsukuba Universities, Japan
Data accessibility	Data is provided in Supplementary materials directly with this article.

Value of the data

- The three-dimensional human embryonic brain was precisely visualized from MRI data
- The development of the brain was effectively demonstrated using the same scale
- The non-uniform thickness of the brain tissue during development was visualized
- The unique and complicated differentiation of the rhombencephalon was shown

1. Data, experimental design, materials and methods

The morphogenesis of the cerebral vesicles and ventricles was analyzed using images derived from 101 human embryo specimens between Carnegie stage 13 and 23 from the Kyoto Collection, which were acquired with a magnetic resonance microscope equipped with a 2.35-T superconducting magnet. The morphology as well as morphometric analysis are provided elsewhere [1].

1.1. MR images of human embryo specimens from Kyoto Collection

Approximately 44,000 human embryos comprising the Kyoto Collection are stored at the Congenital Anomaly Research Center of Kyoto University [2,3]. In most of these cases, pregnancy was terminated during the first trimester for socioeconomic reasons under the Maternity Protection Law of Japan. In the laboratory, aborted embryos were measured, examined, and staged using the criteria provided by O'Rahilly and Müller [4]. 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 [3]. 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 [5]. 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 $40 \mu\text{m}^3$ to $150 \mu\text{m}^3$. 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 [5], the image intensity of the T_1 -weighted images of the human embryos has a close correlation with that of Nissl staining sections.

The 101 selected embryos were re-examined by two authors (T.N. and T.T.) based on previously described criteria [6]. 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.

1.2. 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).

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 filter module of the Amira™ software program named surface color mapping by thickness (the thickness of the brain was visualized on the surface with a color scale).

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2015.05.001>.

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3D models related to the publication: Morphology of the human embryonic brain and ventricles

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* corresponding author: Dr. Tetsuya Takakuwa (tez@hs.med.kyoto-u.ac.jp)

Abstract: The present 3D Dataset contains the 3D models analyzed in : Shiraishi N et al. Morphology and morphometry of the human embryonic brain: A three-dimensional analysis, *NeuroImage*, 115, 2015, 96-103, DOI: [10.1016/j.neuroimage.2015.04.044](https://doi.org/10.1016/j.neuroimage.2015.04.044).

Key words: human brain, human embryo, magnetic resonance imaging, three-dimensional reconstruction

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SPECIMEN LIST

The morphogenesis of the cerebral ventricles was visualized using images derived from human embryo specimens between Carnegie stage (CS) 13 and 23 from the Kyoto Collection (Congenital Anomaly Research Center, Graduate School of Medicine, Kyoto University), which were acquired with a magnetic resonance microscope equipped with a 2.35-T superconducting magnet.

Specimen ids	Species	Developmental Stage
M3#24_KC-CS13BRN50455	<i>Homo sapiens</i>	Carnegie stage 13
M3#25_KC-CS14BRN18834	<i>Homo sapiens</i>	Carnegie stage 14
M3#26_KC-CS15BRN19975	<i>Homo sapiens</i>	Carnegie stage 15
M3#27_KC-CS16BRN7870	<i>Homo sapiens</i>	Carnegie stage 16
M3#28_KC-CS17BRN26702	<i>Homo sapiens</i>	Carnegie stage 17
M3#29_KC-CS18BRN25914	<i>Homo sapiens</i>	Carnegie stage 18
M3#30_KC-CS19BRN16508	<i>Homo sapiens</i>	Carnegie stage 19
M3#31_KC-CS20BRN26581	<i>Homo sapiens</i>	Carnegie stage 20
M3#32_KC-CS21BRN33434	<i>Homo sapiens</i>	Carnegie stage 21
M3#33_KC-CS22BRN27960	<i>Homo sapiens</i>	Carnegie stage 22
M3#34_KC-CS23BRN28189	<i>Homo sapiens</i>	Carnegie stage 23

METHODS

Well-preserved human embryos between CS 13 and CS 23 were selected from Kyoto Collection for MR microscopic imaging (Nishimura et al, 1968; Shiota et al, 2007; O’Rahilly and Müller, 1987). 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 brain tissue and ventricles were segmented for 3D reconstruction using FSL View of FMRIB Software LibraryTM (ver. 4.1.9, Analysis Group, FMRIB, Oxford, UK). Three-dimensional morphology of the brain tissue and

ventricles were computationally reconstructed with AmiraTM software (ver. 5.4.0, Visage Imaging, Berlin, Germany). The 3D surface models were then processed with ISE-MeshTools (Lebrun, 2014); each model was orientated, tagged and labelled using this software. All tagged surfaces are provided in .vtk format, and labels in .flg format. All 3D surface models are also provided in .ply format, and can therefore be opened with a wide range of freeware. Representative lateral view of the brain tissue and ventricles are shown in Figure 1.

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

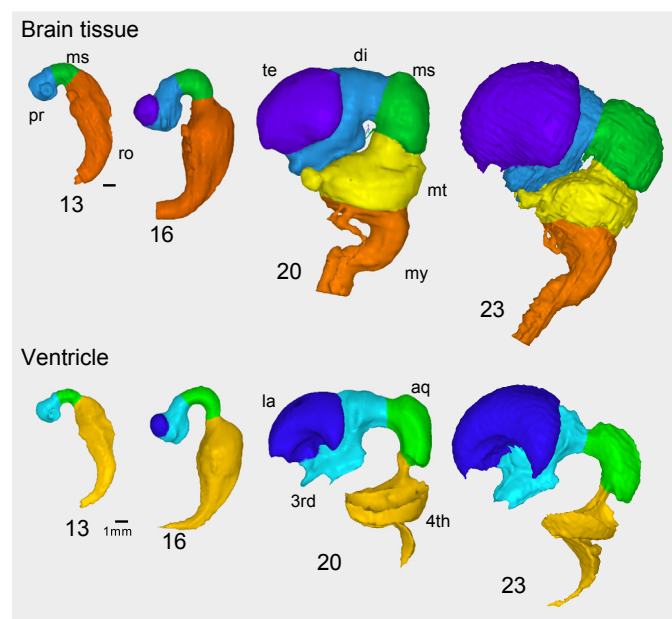


Figure 1: Representative lateral view of the brain tissue and ventricles. pr; prosencephalon, ms; mesencephalon, ro; rhombencephalon, te; telencephalon, di; diencephalon, mt; metencephalon, my; myelencephalon, la; lateral ventricle, 3rd; third ventricle, aq; aqueduct of midbrain, 4th; fourth ventricle.

analysis, *NeuroImage*, 115, 2015, 96-103, DOI: [10.1016/j.neuroimage.2015.04.044](https://doi.org/10.1016/j.neuroimage.2015.04.044)

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