

# **Developmental patterns of chimpanzee cerebral tissues provide important clues for understanding the remarkable enlargement of the human brain**

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## SUMMARY

Developmental prolongation is thought to contribute to the remarkable brain enlargement observed in modern humans (*Homo sapiens*). However, the developmental trajectories of cerebral tissues have not been explored in chimpanzees (*Pan troglodytes*), even though they are our closest living relatives. To address this lack of information, the development of cerebral tissues was tracked in growing chimpanzees during infancy and the juvenile stage using three-dimensional magnetic resonance imaging and compared to that of humans and rhesus macaques (*Macaca mulatta*). Overall cerebral development in chimpanzees demonstrated less maturity and a more protracted course during prepuberty, as observed in humans but not in macaques. However, the rapid increase of cerebral total volume and proportional dynamic change of the cerebral tissue in humans during early infancy, when white matter volume increases dramatically, did not occur in chimpanzees. A dynamic reorganization of cerebral tissues of the brain during early infancy, driven mainly by enhancement of neuronal connectivity, is likely to have emerged in the human lineage after the split between humans and chimpanzees and to have promoted the increase of brain volume in humans. Our findings may lead to powerful insights into the ontogenetic mechanism underlying human brain enlargement.

**Keywords:** brain evolution; brain development; chimpanzees; encephalization; infancy; magnetic resonance imaging (MRI)

## 1. INTRODUCTION

The brain size of humans has increased dramatically during the evolution of *Homo* [1-5]. As a result, although brain size in primates is primarily related to body size, the human brain is approximately three times larger than expected for a primate of the same body weight, a process called encephalization [6]. Neuroanatomical studies show that the number of neurons and glia/neuron ratio of the human brain do not deviate from what would be expected from a primate brain of similar body weight, implying that the human brain conforms to a scaled-up primate brain [7, 8]. However, studies comparing humans to nonhuman primates reveal that human brain evolution has consisted of not merely an enlargement, but rather has involved changes at all levels of brain structure. These include the cellular and laminar organization of cortical areas [9-12]. Therefore, elucidating the differences in the ontogenetic mechanism underlying brain structure between humans and nonhuman primates will provide important clues to clarify the remarkable brain enlargement observed in modern humans.

Over the last century, studies of comparative primate morphology led to the proposal that prolongation of the high foetal developmental rate after birth [13-16] and extension of the juvenile period [17-21] were essential to promote the remarkable brain enlargement of modern humans and the emergence of human-specific cognitive and behavioural traits. Recently, a highly cited study obtained the brain size growth profile of primates from a number of preserved brain samples and concluded that rapid growth velocity of the brain in the early postnatal stage rather than prolongation of the developmental period contributes to the brain enlargement observed in humans [22]. However, confounding factors inherent to

using preserved brain samples to capture the true ontogenetic brain pattern (e.g., individual variation and abnormality/pathology resulting in early death) raise concerns about the robustness of this conclusion [22]. More importantly, comprehensively and quantitatively elucidating the ontogenetic changes in brain tissues is important to verifying the ontogenetic modulation hypothesis of human encephalization from the perspective of brain structural reorganization processes.

Recently, an increasing number of studies have utilised three-dimensional magnetic resonance imaging (MRI) to determine ontogenetic changes to grey matter (GM) and white matter (WM) volumes in humans [23-27] and monkeys [28-30]. However, the underlying ontogenetic process governing the remarkable brain enlargement observed in modern humans remains unclear, because the developmental trajectory of the WM and GM volumes has not been explored in our closest living primate relatives, the chimpanzees. To address this lack of information and uncover empirical evidence for the remarkable enlargement of the human brain during the postnatal period, we tracked the development of the cerebral tissues in growing chimpanzees from infancy to the juvenile period using three-dimensional MRI and compared these results with previously recorded data from humans and rhesus macaques. Our findings reveal common features of the developmental trajectory of brain tissues between the hominoids (humans and chimpanzees), as well as unique features of humans.

## **2. MATERIAL AND METHODS**

### **(a) *Measurement of age-related volumetric changes in chimpanzees***

#### **(i) *Participants***

Three growing chimpanzees, named Ayumu (male), Cleo (female), and Pal (female), and two adult chimpanzees, named Reo (male) and Ai (female), participated in this study. All subjects lived within a social group of 14 individuals in an enriched environment at the Primate Research Institute, Kyoto University (KUPRI) [31, 32]. Our three young chimpanzees were born on 24 April, 2000, 19 June, 2000, and 9 August, 2000, respectively. The treatment of the chimpanzees was in accordance with the 2002 version of the Guidelines for the Care and Use of Laboratory Primates issued by KUPRI. All protocols were approved by the Committee for the Care and Use of Laboratory Primates of KUPRI.

(ii) *Image acquisition*

Three-dimensional T1-weighted whole brain images were acquired from the three growing chimpanzees, when ages ranged from 6 months to 6 years, with a 0.2-Tesla MR imager (Signa Profile; General Electric) using the same three-dimensional spoiled-gradient recalled acquisition in steady state (SPGR) imaging sequence. For comparison, adult data were obtained from two chimpanzees. Prior to scanning, the three growing and two adult chimpanzees were anaesthetised with ketamine (3.5 mg/kg) and medetomidine (0.035 mg/kg), and then transported to the MRI scanner. The subjects remained anaesthetised for the duration of the scans and during transportation between their home cage and the scanner (total time anaesthetised approximately 2 h). They were placed in the scanner chamber in a supine position with their heads fitted inside either the extremity (for the growing chimpanzees) or the head coil (for the adult chimpanzees) (figure 1a). The three-dimensional SPGR acquisition sequence was obtained with the following acquisition parameters: repetition time (TR): 46 ms; echo time (TE): 10 ms; flip angle: 60°; slice

thickness: 1.0 mm; field of view: 14–16 cm (for the growing chimpanzees) or 24 cm (for the adult chimpanzees); matrix size,  $256 \times 256$ ; number of excitations: two.

(iii) *Image processing*

The MR images for each individual were analysed using the following series of manual and automated procedures. (1) All images were analysed using Analyze 9.0 software (Mayo Clinic, Mayo Foundation, Rochester, MN, USA) and converted to cubic voxel dimensions of 0.55 mm using a cubic spline interpolation algorithm. (2) Brain image volumes were realigned to a standard anatomical orientation with the transaxial plane parallel to the anterior commissure-posterior commissure line and perpendicular to the interhemispheric fissure. (3) The cerebral portion of the brain was semi-manually extracted using Brain Extraction Tool (BET) [33] in the FSL package (version 4.1; [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)) [34]. Nonbrain tissues (scalp, orbits) were removed, followed by cerebellar and brain stem tissues (midbrain, pons, medulla). Noncerebral tissues were removed in the coronal plane, starting at the most posterior point and proceeding anteriorly until no obvious break was evident between the midbrain and thalamus [35, 36]. Next, noncerebral tissues were removed in the axial plane according to the method of previous studies [35, 36], starting at the most inferior slice and proceeding superiorly until no obvious break was evident between the midbrain and the posterior limb of the internal capsule (the transition between the cerebral peduncle and the posterior limb of the internal capsule). Thus, the cerebral portion included most of the deep central grey matter (GM) (caudate nuclei, putamen, globus pallidus, lentiform nuclei, thalamus, and intervening white matter [WM]), the hippocampus, and the amygdala in all subjects. (4) MRI data were spatially smoothed using

Smallest Univalued Segment Assimilating Nucleus (SUSAN) [37] in FSL, which reduces noise, without blurring the underlying images. (5) Each brain volume was segmented into GM, WM, and cerebrospinal fluid (CSF) based on signal intensity, and magnetic field inhomogeneity was corrected using FMRIB's Automated Segmentation Tool (FAST) [38] in FSL (figure 1b). This method was based on a hidden Markov random field model and an associated expectation-maximization algorithm. A sample of the results of tissue segmentation at each developmental stage, particularly in infancy, were reviewed by a neuroradiologist (H.T.) to determine if the GM/WM borders determined by FAST were accurate. Next, all the results of the GM and WM segmentation were reviewed and corrected semi-automatically when necessary. (6) The absolute volumes of GM and WM in the cerebrum were measured. The volumes of the cerebrum were calculated from an automatic count of the number of voxels per  $\text{mm}^3$  using FSLUTILS in FSL (table S1). The total volume of the cerebrum corresponded to the sum of GM and WM volumes of the cerebrum.

Two image analysts (T.S. and H.M.), who were blinded to the sex and age of the subjects, semi-manually traced and measured the entire cerebrum. T.S. identified the landmarks of the cerebrum in all brain images in consultation with a neuroradiologist (H.T.) and anatomical experts (A.M. and M.M.). An inter-rater reliability analysis was conducted to compare the cerebral measurements obtained by T.S. with a sample of brain scans measured by H.M. Ten brain scans were randomly selected for analysis. The Pearson's correlation coefficient for the comparison of the results obtained by T.S. and H.M. was  $r = 0.91$ ,  $P < 0.01$ .

(b) *Comparison of the developmental trajectories of chimpanzee, human, and rhesus macaque brain tissue volumes*

Direct comparison of the developmental trajectories of the chimpanzees with those in humans and rhesus macaques allowed the identification of features shared across humans, chimpanzees, and macaques; hominoid (human and chimpanzee)-shared features; and human-specific features. In statistical analyses, the same procedures were used to analyse data from chimpanzees, humans, and macaques.

(i) *Humans*

Human cross-sectional data of age-related brain volume from 28 healthy Japanese children (14 males, 14 females), whose ages ranged from 1 month to 10.5 years (see details in [24]) were analysed. The comparison to human adult volumes was based on the data from 16 healthy adults who served as controls (M. Matsui, C. Tanaka, L. Niu, J. Matsuzawa, K. Noguchi, T. Miyawaki, W. B. Bilker, M. Wierzbicki and R. C. Gur 2010, unpublished data; these data were presented as an abstract entitled ‘age-related volumetric changes of prefrontal grey and white matter from healthy infants to adults’ at the twentieth annual Rotman Research Institute Conference, ‘Frontal Lobes’). Adult subject characteristics were as follows: mean (SD) age, 21.3 (1.8) years; female/male ratio, 50% male. All parents and adult participants gave written informed consent for participation after the nature and possible consequences of the study were explained. All protocols of the study were approved by the Committee on Medical Ethics of Toyama University.

(ii) *Rhesus macaques*

Macaque longitudinal data of age-related brain volume from six normal rhesus macaques (4 males, 2 females), whose ages ranged from 3 months and 4 years, were analysed (see details in [28]). The macaque subjects were raised by experienced veterinary nursery staff and were also placed for several hours daily in a social group with several other animals of the same age. These rearing conditions have proved optimal for the development of social relationships in infant macaques separated from their mothers near birth, as compared with rearing without conspecifics or pair-rearing with several rotating partners. The treatment of the macaques was in accordance with the NRC Guide for Care and Use of Laboratory Animals, and the animal protocol was approved by the Institutional Animal Care and Use Committee of the National Institute of Mental Health (NIMH).

Unlike in the chimpanzee and human studies, the ventricular system was included in the cerebrum in the macaque study [28]. Moreover, the estimation of GM volume in the macaque study (not previously published) differed somewhat from the GM volume estimation in the chimpanzee and human studies. GM volume in macaques was calculated by subtracting the WM volume from the total volume, including the ventricular volume, while those in chimpanzees and humans were calculated by subtracting the WM volume from the total volume, not including the ventricular volume [28]. No significant age-related changes in the total amount of cerebrospinal fluid in the ventricles and external space surrounding the brain were found in a previous study in rhesus macaques [29]. Therefore, developmental changes in the estimated GM of the macaque cerebrum in this study were considered to parallel those of the real GM of the macaque cerebrum. A more detailed

description of the demarcation of the cerebral tissues and the different types of datasets in humans and rhesus macaques is included in the electronic supplementary material.

**(c) *Definitions of developmental stages in chimpanzees, humans, and rhesus macaques***

In this study, developmental indicators were chosen based on a combination of dental eruption and sexual maturation for inter-species comparisons. In the developmental stages based on dental eruption, three developmental stages were defined: “early infancy”, “late infancy”, and “juvenile” (figure S1) [39-41]. These stages were demarcated by the eruption of the first deciduous tooth and the eruption of the first permanent tooth. The juvenile stage ends at sexual maturation (menarche, first ejaculation) [42-45]. The developmental stages analysed were, in chimpanzees, ~1 year of age, ~3 years of age, and ~8 years of age; in humans, ~2 years of age, ~6 years of age, and ~12 years of age; and in macaques, ~0.4 years of age, ~1.3 years of age, and ~3.2 years of age.

**(d) *Statistical analysis***

All statistical analyses were performed using SPSS 19 (SPSS, Chicago) and R 2.11.1 (<http://www.r-project.org/>) software. Hypothesis tests for model building were based on  $F$  statistics. All statistical hypothesis tests were conducted at a significance level of 0.05.

**(i) *Total and tissue volumes of the cerebrum.***

F tests were used to determine whether the order of a developmental model was cubic, quadratic, or linear. First, linear, quadratic, or cubic polynomial regression models were fitted by age using SPSS 19 to identify the brain volume development patterns in the cerebrum. If a cubic model did not yield significant results, a quadratic model was tested; if a quadratic model did not yield significant results, a linear model was tested. Thus, a

growth model was polynomial /nonlinear if either the cubic or quadratic term significantly contributed to the regression equation. The Akaike information criterion (a log-likelihood function) [46] was used to ensure effective model selection.

Second, using R 2.11.1 software, the data that showed nonlinear trajectories was fitted by locally weighted polynomial regression [47]. In this way, even with relatively few data points, gestational age-related volume changes could be delineated by applying the curve fitting suggested by previous human studies [48, 49] and a previous chimpanzee study [36], without enforcing a common parametric function on the data set, as is the case with linear polynomial models. The fit at a given age was made using values in a neighbourhood that included a proportion,  $\alpha$ , and for  $\alpha < 1$ , the neighbourhood included a proportion,  $\alpha$ , of the values. Data were fitted in four interactions with  $\alpha = 0.70$ . The observed and fitted values of the total, WM, and GM volumes in the cerebrum were plotted as a function of age to display the age-related change.

To assess the differences in the developmental patterns of the total, GM, and WM volumes in the cerebrum among chimpanzees, humans, and macaques, the relative total, GM, and WM volumes were calculated as a percentage of the adult volumes in the cerebrum. To adequately describe the variability in the data among adult chimpanzees compared to that among young chimpanzees, data on the GM and WM volumes of the cerebrum from six adult chimpanzees used in a previous study [35] were added to the present data from the two adult chimpanzees.

(ii) *The increase of GM relative to WM*

The differences in the postnatal developmental patterns of the total volume of the cerebrum between chimpanzees, humans, and macaques appears likely to be due to differences in the developmental patterns of brain tissues during the postnatal period, and these differences greatly influence the ultimate difference in the adult brain volume size among the three species. Therefore, to elucidate species-specific variations in chimpanzees, humans, and macaques, the relative growth of the GM versus the WM of the developing cerebrum was evaluated and compared to the adult value. The relative growth of the GM versus the WM was calculated and compared to the adult value by dividing the ratio of GM volume to WM volume in the cerebrum by the adult ratio.

### **3. RESULTS**

#### **(a) *Total and tissue volumes***

The results of brain tissue segmentation revealed noteworthy developmental changes in chimpanzees over the course of the study period (figure 1*b* and figure S1). The increase of total cerebral volume during early infancy and the juvenile stage in chimpanzees and humans was approximately three times greater than that in macaques. The total volume of the chimpanzee cerebrum increased 32.4% over the developmental period from the middle of early infancy to the second half of the juvenile stage (6 months to 6 years) (figure 2*a*). The corresponding value in the human cerebrum during approximately the same developmental period (1 year to 10.5 years) was 27.7% (figure 2*b*). By contrast, the total volume of the macaque cerebrum increased only 10.9% during approximately the same developmental period (3 months to 2.7 years; figure 2*c*). A more detailed description of the

total and tissue volumes in chimpanzees, humans, and macaques is available in the electronic supplementary material, table S1, table S2, table S3, and table S4.

Chimpanzees and humans demonstrated a nonlinear developmental course of the GM and WM volumes and a common rate of increase of these tissue volumes from early infancy through the juvenile stage. The GM and WM volumes of the chimpanzee cerebrum increased by 10.0% and 92.5%, respectively, from the middle of early infancy to the second half of the juvenile stage (6 months to 6 years) (figure 2a). The respective values in the human cerebrum during the corresponding developmental period were 6.7% and 96.7% (figure 2b). By marked contrast, in rhesus macaques, no significant increase of GM volume occurred during approximately the same developmental period (3 months to 2.7 years) (figure 2c). Moreover, the increase of WM volume in the macaque cerebrum during this developmental period was 74.7%, which was smaller than that the increase in WM volume in chimpanzees and humans (figure 2c).

**(b) *Higher rate of total cerebrum volume accumulation in human infants***

Chimpanzees and humans differed from macaques in showing less maturity of brain volume after birth and prolonged development of the total and WM volumes of the cerebrum. The total and WM volumes in chimpanzees at the middle of early infancy (6 months) were 73.8% and 36.5% of the adult volume, respectively (figure 3a). The corresponding values in humans at approximately the same developmental period (1 year) were 74.2% and 40.5%, respectively (figure 3b). By contrast, the total cerebral volume of macaques had already reached a plateau at the middle of early infancy (3 months) (figure

3c). The cerebral WM volume of macaques reached 51.2% of the adult volume at the middle of early infancy (figure 3c).

Interestingly, the rate of increase in total volume of the chimpanzee cerebrum during early infancy was only half that of humans, although both chimpanzees and humans exhibited immaturity of the total volume at early infancy and a relatively protracted development of the total volume compared with macaques during early infancy and the juvenile stage. The total volume of the chimpanzee cerebrum increased by 8.4% from the middle of early infancy until the end of early infancy (6 months to 1 year) (figure 2a), while the total volume of the human cerebrum increased by 16.4% during approximately the same developmental period (1 year to 2 years) (figure 2b). By contrast, the total volume of the macaque cerebrum increased by only 1.6% during approximately the same developmental stage (3 months to 4.8 months) (figure 2c).

This great difference in the developmental patterns of the total volume of the cerebrum at early infancy between chimpanzees and humans appears to be caused by differences in the developmental patterns of brain tissues during this stage and to greatly influence the ultimate difference in the adult brain volume between the two species. To verify this possibility, we attempted to evaluate the relative growth of the GM versus the WM of the developing chimpanzee cerebrum. We then compared the results to the adult value and to those of humans and macaques. The proportion of GM relative to WM was calculated by dividing the ratio of GM volume to WM volume in the cerebrum at a given developmental stage by the adult ratio.

Like humans, chimpanzees substantially differed from macaques in the proportions of brain tissues of the cerebrum at an early developmental stage. At the middle of early infancy (6 months), the proportion of GM relative to WM of the cerebrum in chimpanzees was 3.51 (figure 4a). The corresponding value in humans at approximately the same developmental stage (1 year) was 3.29 (figure 4b). By contrast, the proportion of GM relative to WM of the macaque cerebrum at approximately the same developmental stage (3 months) was only 1.93 (figure 4c).

However, the proportion of GM relative to WM of the cerebrum in chimpanzee infants developed along a slower trajectory during early infancy compared with that in human infants. The proportion of GM relative to WM of the chimpanzee cerebrum changed from 3.51 to 3.18 from the middle of early infancy to the end of early infancy (6 months to 1 year) (figure 4a). By marked contrast, in humans, the proportion changed from 3.29 to 2.05 during approximately the same developmental stage (1 year to 2 years) (figure 4b). In macaques, the proportion of GM relative to WM of the cerebrum changed only from 1.93 to 1.82 during approximately the same developmental stage (3 months to 4.8 months) (figure 4c). These results suggest that human infants exhibit a more dynamic proportional change in brain tissues during early infancy. A more detailed description of the time course of changes in the proportion of GM relative to WM of the cerebrum in chimpanzees, humans, and macaques is included as electronic supplementary material and in table S5.

Although we observed that GM and WM volumes of the cerebrum increased during early infancy both in chimpanzees and humans, we demonstrated that this difference is attributable to differences between the species in the rate of WM volume increase during

this developmental stage. The rate of WM volume increase in the chimpanzee cerebrum during early infancy was lower than that in the human cerebrum, while the rate of GM volume increase in the chimpanzee cerebrum at this developmental stage was almost the same as that in human infants. The GM and WM volumes of the chimpanzee cerebrum increased by 5.2% and 17.2%, respectively, over the developmental period from the middle of early infancy to the end of early infancy (6 months to 1 year) (figure 2a). By contrast, the corresponding values increased to 8.4% and 42.8%, respectively, during approximately the same developmental period (1 year to 2 years) in humans (figure 2b). In macaques, no significant age-related change in the GM volume of the cerebrum occurred during the study period (3 months to 4 years) (figure 2c). The WM volume of the macaque cerebrum increased only by 9.4% from the middle of early infancy to the end of early infancy (3 months to 4.8 months) (figure 2c).

#### **4. DISCUSSION**

We succeeded in empirically verifying the previously proposed hypothesis concerning the ontogenetic mechanism underlying the remarkable brain enlargement in modern humans. Despite the relatively small sample size, our results revealed that overall cerebral development in chimpanzees followed a less mature and more protracted course during prepuberty, as observed in humans but not in macaques. However, a rapid increase of the cerebral total volume during early infancy did not occur in chimpanzees. Therefore, our findings support the hypothesis of a previous study based on preserved brain samples that the rapid brain development rate in the early postnatal stage rather than the extension of the developmental period contributes to the enlargement of the human brain [22]. Moreover,

these findings suggest that dynamic changes in the proportions of human brain tissues, driven mainly by an increase of WM during early infancy, may promote the enlargement of the human brain.–

From the results of this brain imaging study alone, it is difficult to draw firm conclusions regarding the cellular changes involved in the dynamic maturational processes involved. However, the increase in GM volume during the postnatal period is presumed to reflect the increase of dendrites and axons as well as glial cells, which are crucial to the formation, operation, and maintenance of neural circuits [25, 50]. The data used in the present study included subcortical GM such as the basal ganglia in the three species. The GM of the basal ganglia typically decreases in volume over the course of development in humans [51]. In this context, the decrease of subcortical GM volume after birth seemed to influence the developmental changes in total GM volume of the cerebrum in humans and chimpanzees in this study.

The increase in WM volume is consistent with the results of postmortem studies showing that maturational changes are accompanied by myelination, which improves the conduction speed of fibres between different brain regions [52, 53]. Interestingly, the process of WM development after birth is expected to provide powerful insights into the evolutionary history of human brain structure and function. Recent imaging studies of human brain development confirmed a positive correlation between structural and functional connectivity in WM maturation and demonstrated that this relationship strengthened with age [54-56]. Furthermore, the refinement of neural networks mediated by WM maturation promotes increased connection efficiency throughout the brain by

continuously increasing integration and decreasing segregation of structural connectivity with age [55]. Thus, our results suggest that the enhancement of the neural connectivity between brain regions and the construction of the neural circuits observed during the postnatal period were established in the ancestral lineage of chimpanzees and modern humans after its divergence from that of macaques. However, the lineage leading solely to modern humans must have undergone dramatic changes in connectivity to explain the dynamic reorganization of human brain tissues that occurs during infancy.

Moreover, a recent comparative neuroanatomical study shows that the developmental trajectory of neocortical myelination in humans is distinct from that in chimpanzees [57]. In chimpanzees, the density of myelinated axons increased until adult-like levels were achieved at approximately the time of sexual maturity [57]. In contrast, humans show a prolonged increase of myelination beyond late adolescence [57]. Thus, as the next step of our ongoing longitudinal MRI study, we will trace the developmental trajectory of the WM volume of the chimpanzee cerebrum after puberty and compare it with that of the human cerebrum in order to determine whether the enhancement of the neural connectivity of the cerebrum continues beyond puberty and adolescence at the neuroimaging level,.

Importantly, several recent studies have suggested that the period from birth to two years, corresponding to early infancy, is a critical period of postnatal brain development in humans from the perspectives of brain structures resulting from increased brain volume [24, 58]; elaboration of new synapses, myelination [59] and dendrites [60]; and the brain's default network [54]. Moreover, children placed in foster care before the age of two appear to make far better improvements in cognitive development than those placed in foster care

after the age of two [61]. Our finding of a rapid increase in the volume of the human cerebrum during the first two years after birth, a process that results in the dynamic reorganization of brain tissue, complements previous findings on human neurodevelopment and human cognitive development from the standpoint of human brain ontogenetic patterns.

Collectively, our results suggest that prolonged development of the cerebrum at postnatal developmental stages existed in the last common ancestor of chimpanzees and humans. However, the dynamic developmental changes in the human brain tissues, mainly driven by the elaboration of neural connections, may have emerged in the human lineage after the split between humans and chimpanzees and may have promoted the evolutionary enlargement of the modern human brain. These findings point to the existence of an ontogenetic mechanism for the remarkable brain enlargement observed in modern humans. Furthermore, the information obtained in this study via a direct comparison of the developmental trajectories of brain tissues of three primate species highlights the importance of focusing on early infant development for understanding the patterns of brain development and changes in cognition in human children.

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## FIGURE CAPTIONS

Figure 1. An ontogenetic series of MRI images of the whole cerebrum in a chimpanzee

brain during early infancy and the juvenile stage. (a) MRI scanning of the brain of a

chimpanzee infant (Ayumu) at 6 months of age. (b) MRI brain images aligned by age are

shown for a representative young chimpanzee (Pal) and an adult chimpanzee (Reo) for

comparison. (i) T1-weighted anatomical brain images. (ii) Segmentation of the cerebrum:

grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF). (iii)

Three-dimensional renderings of the cerebrum from superior and right and left lateral views.

The coloured bar to the left of the images indicates the developmental stage based on dental

eruption and sexual maturation. The indicated developmental stages in chimpanzees are

early infancy (magenta), late infancy (yellow), juvenile (green), and adult stage (purple).

Figure 2. Evaluation of total, GM, and WM volumes in the cerebrum during early infancy

and the juvenile stage. Age-related changes in the total, GM, and WM volumes in the

cerebrum are shown for (a) chimpanzees (Ayumu, Cleo, and Pal), (b) humans ( $n = 28$ ), and (c) rhesus macaques ( $n = 6$ ). To compare the developmental trajectory of GM volume in rhesus monkey with that of chimpanzees and humans, the estimation of GM volume in rhesus macaques was calculated by subtracting the WM volume from the total volume, including the ventricular volume. The coloured bar below the graphs indicates the developmental stage based on dental eruption and sexual maturation. The indicated developmental stages are early infancy (magenta), late infancy (yellow), juvenile (green), and puberty (blue). When no evidence of a significant effect of age on the estimation of brain volume was detected, no regression line was fitted. See also [24] and [28] for more details of the human and rhesus macaque data, respectively.

Figure 3. Evaluation of total, GM, and WM volumes relative to the adult volumes in the cerebrum during early infancy and the juvenile stage. Age-related changes in total, GM, and WM volumes relative to the adult volumes in the cerebrum are shown for (a) chimpanzees (Ayumu, Cleo, and Pal), (b) humans ( $n = 28$ ), and (c) rhesus macaques ( $n = 6$ ). The coloured bar below the graphs indicates the developmental stage based on dental eruption and sexual maturation. The indicated developmental stages are early infancy (magenta), late infancy (yellow), juvenile (green), and puberty (blue). When no evidence of a significant effect of age on the estimation of brain volume was detected, no regression line was fitted.

Figure 4. Evaluation of the proportion of GM volume to WM volume in the cerebrum during early infancy and the juvenile stage with that in adults. Age-related changes in the growth velocity of total and tissue volumes in the cerebrum are shown in (a) chimpanzees

(Ayumu, Cleo, and Pal), (b) humans ( $n = 28$ ), and (c) rhesus macaques ( $n = 6$ ). The coloured bar below the graphs indicates the developmental stage based on dental eruption and sexual maturation. The indicated developmental stages are early infancy (magenta), late infancy (yellow), juvenile stage (green), and puberty (blue). When no evidence of a significant effect of age on estimation of brain volume was detected, no regression line was fitted.



Figure 2

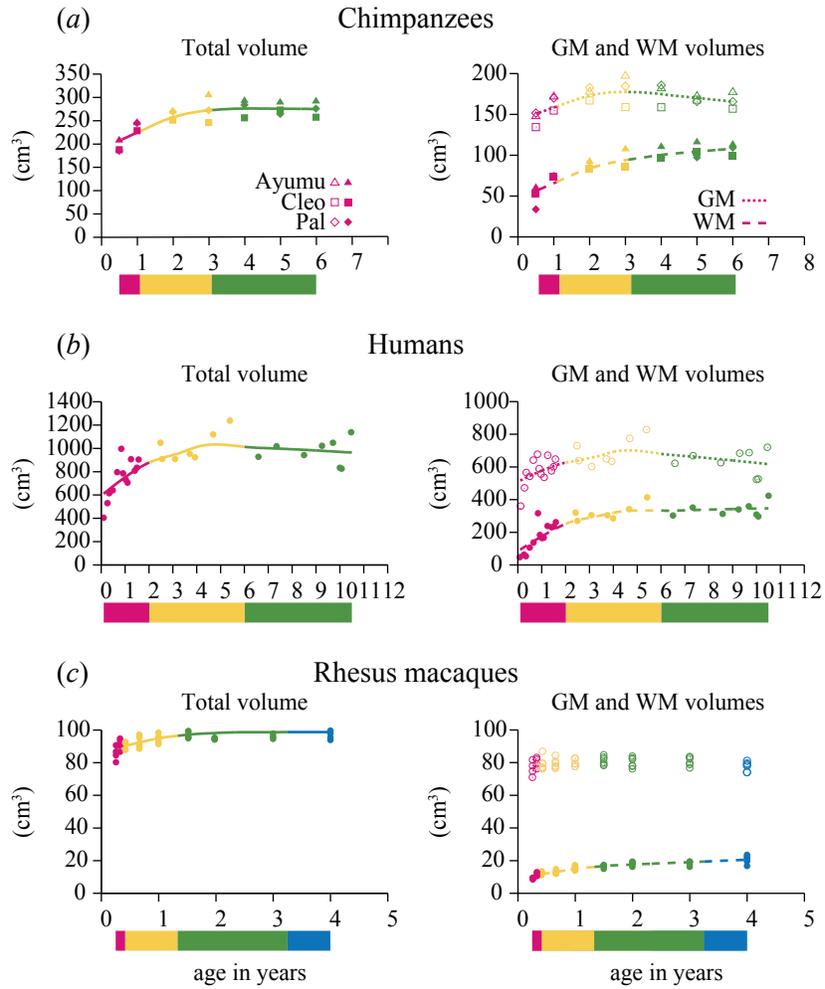


Figure 3

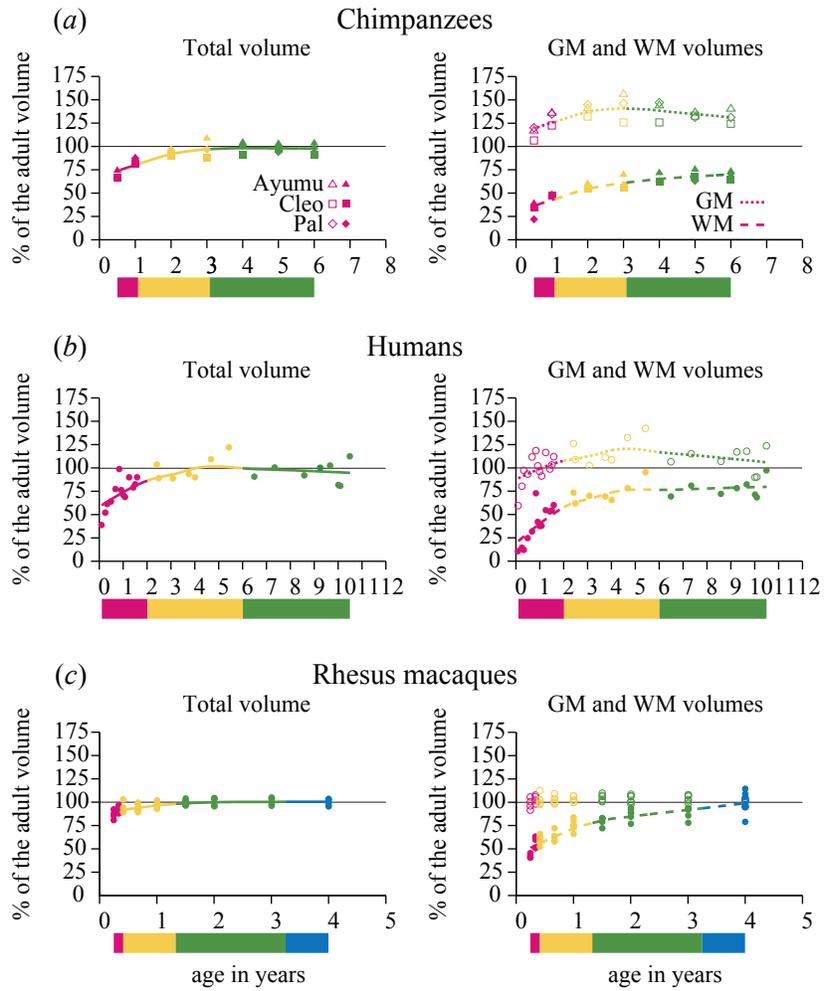
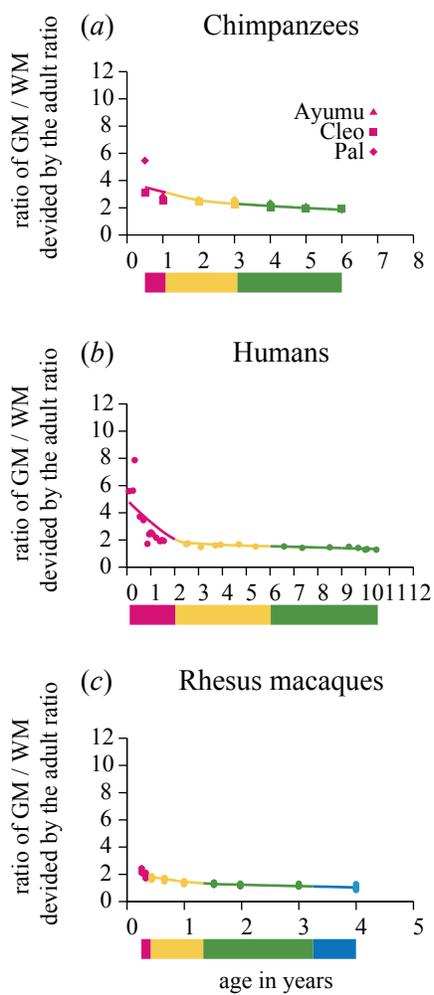


Figure 4



## Electronic supplementary material

### Developmental patterns of chimpanzee cerebral tissues provide important clues for understanding the remarkable enlargement of the human brain

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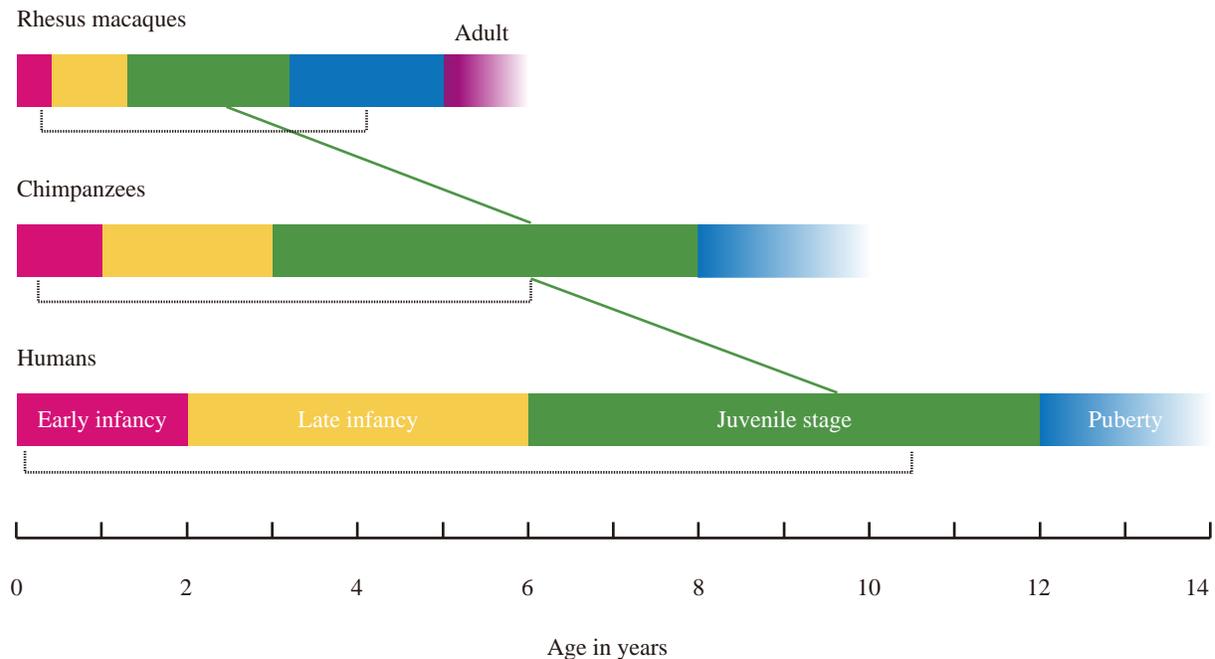


Figure S1. Developmental stages based on combined dental eruption and sexual maturation in chimpanzees, humans, and rhesus macaques. The coloured bars indicate the developmental stages: early infancy (magenta), late infancy (yellow), juvenile (green), puberty (blue), and adult (purple). We compared the developmental trajectories of brain volumes across the three species within the range indicated by the dashed black brackets. The solid green lines represent the developmental stage in humans and macaques corresponding to six years of age (the second half of the juvenile stage) in chimpanzees.

Table S1. Age, total, GM, and WM volumes in chimpanzees

Subject (Sex)	Age (years)	Cerebrum (cm <sup>3</sup> )		
		Total	GM	WM
Ayumu (M)	0.5	207.4	147.4	60.0
	1	244.7	171.5	73.2
	2	269.7	177.5	92.2
	3	304.3	197.0	107.3
	4	291.7	181.4	110.3
	5	288.0	172.0	116.0
	6	289.9	177.0	112.9
Cleo (F)	0.5	187.6	134.4	53.3
	1	228.2	154.7	73.5
	2	250.7	167.0	83.7
	3	245.2	158.9	86.4
	4	255.3	158.8	96.5
	5	271.9	168.1	103.9
	6	256.2	157.0	99.2
Pal (F)	0.5	185.4	151.6	33.8
	1	244.8	170.1	74.7
	2	268.7	182.8	85.9
	3	271.5	184.6	86.9
	4	282.8	185.7	97.1
	5	263.3	165.9	97.4
	6	274.8	165.6	109.2
Adult chimpanzees				
Reo (M)	23	262.178	131.127	131.051
Ai (F)	32	312.307	135.649	176.658
Shoenemann et al. (2005)				
Merv (M)	-	334.7	135.4	199.4
Laz (M)	-	238.0	111.1	126.9
Jimmy (M)	-	263.8	130.4	133.3
Mary (F)	-	271.8	112.2	159.6
Lulu (F)	-	248.1	102.0	146.0
Kengree (F)	-	310.7	152.1	158.6
Average	-	280.2	126.2	154.0
±SD	-	34.7	16.5	25.0

GM, grey matter; WM, white matter.

Table S2. Age, total, GM, and WM volumes in humans

Subject	Age (years)	Total	Cerebrum (cm <sup>3</sup> )	
			GM	WM
No.1	0.1	394.0	346.8	47.2
No.2	0.3	530.2	467.2	62.9
No.3	0.3	622.5	569.0	53.4
No.4	0.5	652.0	544.6	107.3
No.5	0.7	788.1	650.3	137.9
No.6	0.8	1007.0	689.6	317.3
No.7	0.9	778.2	594.6	183.6
No.8	1.0	724.0	560.1	163.9
No.9	1.1	697.7	531.6	166.1
No.10	1.3	915.3	677.3	238.0
No.11	1.4	807.3	575.0	232.3
No.12	1.5	836.7	601.0	235.7
No.13	1.6	914.5	652.1	262.4
No.14	2.4	1055.1	735.4	319.8
No.15	2.5	904.6	635.7	269.0
No.16	3.1	902.3	596.7	305.6
No.17	3.8	952.4	649.2	303.2
No.18	4.0	917.9	632.4	285.5
No.19	4.7	1113.1	771.7	341.4
No.20	5.4	1243.6	830.2	413.4
No.21	6.5	923.4	620.6	302.8
No.22	7.3	1023.4	670.2	353.3
No.23	8.6	938.0	623.8	314.2
No.24	9.3	1022.6	681.5	341.1
No.25	9.7	1043.1	685.5	357.6
No.26	10.0	833.4	523.8	309.6
No.27	10.1	823.1	525.6	297.5
No.28	10.5	1144.0	720.4	423.6

GM, grey matter; WM, white matter. The numerical data for the humans originated in [24].

Table S3. Age, total, GM, and WM volumes in rhesus macaques

Subject	Age (years)	Total	Cerebrum (cm <sup>3</sup> )	
			GM estimation	WM
No.1	0.25	84.2	74.7	9.5
	0.33	94.0	83.4	10.6
	0.42	88.4	76.5	11.9
	0.67	88.1	76.2	11.9
	1.00	92.6	77.5	15.1
	1.50	95.2	78.0	17.2
	2.00	94.3	75.9	18.3
	3.00	98.0	78.7	19.2
	4.00	97.8	74.0	23.8
No.2	0.25	90.9	81.9	9.0
	0.33	91.5	79.1	12.5
	0.42	92.1	79.1	13.0
	0.67	90.9	77.7	13.3
	1.00	91.2	77.5	13.7
	1.50	94.9	79.9	15.0
	2.00	94.2	78.2	16.0
	3.00	96.0	79.8	16.2
	4.00	95.2	78.9	16.3
No.3	0.25	86.5	78.0	8.5
	0.33	95.4	82.2	13.2
	0.42	101.0	87.3	13.7
	0.67	97.7	84.5	13.2
	1.00	100.1	82.7	17.4
	1.50	99.7	83.3	16.4
	2.00	102.9	84.3	18.6
	3.00	102.2	82.7	19.5
	4.00	101.4	81.1	20.3
No.4	0.25	79.5	71.1	8.4
	0.33	86.7	76.1	10.6
	0.42	87.9	75.5	12.4
	0.67	89.2	76.9	12.3
	1.00	96.0	79.4	16.6
	1.50	96.0	78.7	17.3
	2.00	95.1	77.6	17.5
	3.00	94.3	76.6	17.8
	4.00	93.8	74.1	19.7
No.5	0.42	92.8	79.5	13.3
	0.67	95.7	80.7	15.0
	1.00	98.8	83.1	15.6

	1.50	102.3	85.2	17.2
	2.00	101.6	82.5	19.1
	3.00	102.9	83.8	19.1
	4.00	102.2	79.5	22.7
No.6	0.42	87.7	76.9	10.8
	0.67	91.8	79.5	12.3
	1.00	94.5	79.2	15.4
	1.50	99.6	82.4	17.2
	2.00	102.6	83.1	19.5
	3.00	103.3	83.8	19.5
	4.00	100.0	78.1	21.9

GM, grey matter; WM, white matter. The numerical data for the rhesus macaques was taken from [29]. The estimation of GM volume in rhesus macaques (not previously published) was calculated by subtracting the WM volume from the total volume, including the ventricular volume.

Table S4. Results from polynomial regression modelling of developmental trajectories of brain tissue volumes in the cerebrum

	Polynomial regression model			
	Anatomical structure	Best fitting model	F value	P value
Chimpanzees	Total	Cubic	18.89	<u>.0000</u>
	GM	Cubic	7.08	<u>.0027</u>
	WM	Cubic	32.99	<u>.0000</u>
Humans	Total	Cubic	15.93	<u>.0000</u>
	GM	Cubic	5.89	<u>.004</u>
	WM	Cubic	38.15	<u>.0000</u>
Rhesus macaques	Total	Cubic	16.13	<u>.004</u>
	GM	n.s.	2.88	.07
	WM	Cubic	(Quadratic) 32.99	(Quadratic) <u>.0000</u>

GM, grey matter; WM, white matter. Age-related change in total, GM, and WM volume in chimpanzees (Ayumu, Cleo, and Pal), humans (n = 28), and rhesus macaques (n = 6). “Best fitting model”, “F value”, and “P value” indicate the results of the statistical analysis of the age-related changes in brain tissue volumes with a polynomial regression model. The best-fitting model represents the best-fitting model of linear, quadratic, and cubic regression models. Underlined characters indicate Bonferroni-corrected P values < .05 for the model. “n.s.” indicates “not significant”.

Table S5. Results of polynomial regression modelling of the developmental trajectories of the proportion of GM volume to WM volume compared with those adult values in the cerebrum.

	Polynomial regression model		
	Best fitting model	F value	P value
Chimpanzees	Cubic	8.62	<u>.001</u>
Humans	Cubic	16.95	<u>.0000</u>
Rhesus macaques	Cubic	79.88	<u>.0000</u>

GM, grey matter; WM, white matter. Age-related change in the proportion of GM relative to WM volume in chimpanzees (Ayumu, Cleo, and Pal), humans (n = 28), and rhesus macaques (n = 6). “Best fitting model”, “F value”, and “P value” indicate the results of the statistical analysis of the age-related changes in brain tissue volumes with a polynomial regression model. The best fitting model represents the best fitting model of linear, quadratic, and cubic regression models. Underlined characters indicate Bonferroni-corrected P values < .05 for the model.

## 1. SUPPLEMENTARY RESULTS

### (a) *Total and tissue volumes of the cerebrum*

*Chimpanzees.* The total volume of the chimpanzee cerebrum increased nonlinearly from the middle of early infancy to the juvenile stage (six months to 6 years) (figure 2a, table S1, and table S4). The GM and WM volumes of the cerebrum followed nonlinear developmental trajectories during this age period (figure 2a, table S1, and table S4).

*Humans.* The total volume of the human cerebrum increased nonlinearly from around the onset of early infancy to the second half of the juvenile stage (one month to 10.5 years) (figure 2b, table S2, and table S4). The GM and WM volumes of the cerebrum followed nonlinear developmental trajectories during this age period (figure 2b, table S2, and table S4).

*Rhesus macaques.* The total volume of the cerebrum increased nonlinearly during the middle of early infancy until near the onset of the adult stage (three months to 4 years) (figure 2c, table S3, and table S4). The WM volume in the cerebrum also increased nonlinearly during this age period (figure 2c, table S3, and table S4). However, no significant age-related changes in the cerebral GM volume occurred during this period (figure 2c, table S3, and table S4)

### (b) *The increase of GM relative to WM*

*Chimpanzees.* The proportion of GM volume relative to WM volume of the chimpanzee cerebrum increased nonlinearly from the middle of early infancy to the juvenile stage (six months to 6 years) (figure 4a and table S5).

*Humans.* The proportion of GM volume relative to WM volume of the human cerebrum increased nonlinearly from around the onset of early infancy to the second half of the juvenile stage (one month to 10.5 years) (figure 4b and table S5).

*Rhesus macaques.* The proportion of GM volume relative to WM volume of the macaque cerebrum increased nonlinearly from the middle of early infancy until around the onset of the adult stage (three months to 4 years) (figure 4c and table S5).

## 2. SUPPLEMENTARY DISCUSSION

### (a) *Limitations in the demarcation of the cerebral tissues and in the different types of data sets in humans and rhesus macaques*

In this study, although the demarcations of all the cerebral portions in human brains were very similar to those in chimpanzee brains, those of macaque brains were different from those of chimpanzees and humans. Unlike in the chimpanzee and human studies, the ventricular system was included in the cerebrum in the macaque study [28]. Moreover, the method of GM volume estimation in the macaque study (not previously published) differed somewhat from that in the chimpanzee and human studies. GM volume in macaques was calculated by subtracting the WM volume from the total volume, including the ventricular volume, whereas those in chimpanzees and humans were calculated by subtracting the WM volume from the total volume, not including the ventricular volume [28]. However, no significant age-related changes in the total amount of cerebrospinal fluid in the ventricles and external space surrounding the brain were found in a previous cross-sectional study in rhesus macaques [29]. Because the volume of cerebrospinal fluid remained constant, we presumed that the maturational changes in GM volume were not affected by changes in the cerebrospinal fluid. In support of this idea, the same cross-sectional study [29] found no significant age-related changes in GM, a finding that is consistent with the results of the macaque study presented here (see Results). Therefore, developmental changes in the estimated GM of the macaque cerebrum in this study were considered to parallel those of the real GM of the macaque cerebrum.

Furthermore, data sets collected from humans were obtained from cross-sectional imaging studies [24], unlike the data sets collected from chimpanzees and macaques, which were obtained from longitudinal imaging studies. However, these discrepancies are unlikely to appreciably influence the comparison of developmental trajectories of brain tissues among chimpanzees, humans, and macaques, because the volumetric differences that resulted from these discrepancies appear to be subtle. In fact, previous imaging studies that directly compared the developmental patterns of humans and non-human primates indicated that each of these species had characteristic features despite the presence of differences in the anatomical demarcations of the brain, the type of investigation (cross-sectional or longitudinal), and the statistical analysis [28-30]. It is important to ensure that these discrepancies do not lead to contradictory results in future studies. Nonetheless, the present study is the first to directly compare the developmental trajectories of the brain tissue volumes in humans and non-human primates using the same statistical analysis throughout.