Title	Morphometric human embryonic brain features according to developmental stage
Author(s)	Kobayashi, Ami; Ishizu, Koichi; Yamada, Shigehito; Uwabe, Chigako; Kose, Katsumi; Takakuwa, Tetsuya
Citation	Prenatal Diagnosis (2016), 36(4): 338-345
Issue Date	2016-04
URL	http://hdl.handle.net/2433/216674
Right	This is the accepted version of the following article: Kobayashi, A., Ishizu, K., Yamada, S., Uwabe, C., Kose, K., and Takakuwa, T. (2016) Morphometric human embryonic brain features according to developmental stage. Prenat Diagn, 36: 338–345. doi: 10.1002/pd.4786., which has been published in final form at http://dx.doi.org/10.1002/pd.4786. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.; The full-text file will be made open to the public on 21 April 2017 in accordance with publisher's 'Terms and Conditions for Self-Archiving'.; This is not the published version. Please cite only the published version. この論文は出版社版でありません。引用の際には出版社版をご確認ご利用ください。
Type	Journal Article
Textversion	author

Morphometric human embryonic brain features according to developmental stage

[1]Ami Kobayashi, [1]Koichi Ishizu, [1,2]Shigehito Yamada, [2]Chigako Uwabe, [3]Katsumi Kose, [1]Tetsuya Takakuwa

1) Human Health Science, Graduate School of Medicine, Kyoto University, Kyoto, Japan

2) Congenital Anomaly Research Center, Graduate School of Medicine, Kyoto University, Kyoto, Japan

3) Institute of Applied Physics, University of Tsukuba, Ibaragi, Japan

Running title: Human embryonic brain measurements

Word count: 2237 words

Number of Tables; 4, **Figures**; 5

Corresponding author: Dr. Tetsuya Takakuwa, Human Health Science,

Graduate School of Medicine, Kyoto University, 606-8507 Sakyo-ku Shogoin Kawahara-cyo 53, Kyoto, Japan.

E-mail: tez@hs.med.kyoto-u.ac.jp, TEL: +81-75-751-3931

Funding: This study was supported by grants from the Japan Society for the Promotion of Science and the BIRD of Japan Science and Technology Agency (JST); Grant number: #24119002, #25461642, #26220004, #15H01119, #15K15014, #15K08134, #15H05270, #15H01121

Conflicts of interest: There are no conflicts of interest to report.

Bulleted statements

What is already known about this topic?

■ Comparing morphometric data based on embryonic stages and sonographic information can be valuable; however, classic embryology has provided relatively little morphometric data.

What does this study add?

■ Linear, area, and volume measurements of the human embryonic brain according to Carnegie stages were obtained using magnetic resonance microscopy data.

■ All linear measurements, except for bitemporal length (BT) and mesencephalic height (MH), increased non-monotonically.

■ A high correlation between BT and both whole-brain (r = 0.98) and prosencephalon volume (r = 0.99) was found.

Abstract

Objectives: The present study investigated linear, area, and volume measurements of human brain samples according to Carnegie stages (CS) in an attempt to select suitable morphometric features that reflect embryonic development.

Methods: Using magnetic resonance (MR) imaging, we measured seven linear segments, three separate areas, and three regional volumes in 101 samples between CS 13 and CS 23. Brain volume was determined via manual segmentation of the MR image, whereby a formula was generated to estimate the volume of each linear measurement.

Results: All parameters correlated with crown-rump length. Bitemporal length (BT) and mesencephalic height increased linearly according to the CS, and a high correlation between BT and both whole-brain (r = 0.98) and prosencephalon volume (r = 0.99) was found when brain cavity volume was excluded.

Conclusion: Morphometric data related to human embryonic stages are valuable

for correcting and comparing sonographic data. The present approach may contribute to improvements in prenatal diagnostics by enabling the selection of more suitable measurements during early embryonic stages.

Key Words

human brain, human embryo, magnetic resonance imaging, length and area measurements

Abbreviations

Carnegie stage (CS), magnetic resonance (MR), fronto-occipital diameter (FO), bitemporal diameter (BT), mesencephalon length (ML), mesencephalon height (MH), cerebellar length (CL), cerebellar height (CH), biparietal diameter (BPD), crown-rump length (CRL).

Introduction

The use of ultrasound for prenatal diagnostics has rapidly increased over the past 25 years[1-9]. The application of three-dimensional (3D) sonography with high-frequency transvaginal transducers has expanded and now fosters 3D sonoembryology, which provides a basis for assessing normal human development and can also be useful in detecting developmental anomalies. Thus, such technology could contribute to more accurate prenatal diagnoses, as well as enable a shift in the diagnostic time window (from the second to the first trimester). At present, an embryo at 7 gestational weeks (based on the last menstrual period) or younger, which corresponds to a Carnegie stage (CS) of 15–16, can be assessed using morphological and morphometrical analyses. While abnormal embryos younger than 10 weeks' gestation may be observed incidentally, systematic screening using sonographic parameters could result in the detection of abnormalities during the late, first trimester (10–11 weeks' gestation)[1].

The human brain develops in a very complicated manner during the embryonic period[10]. For example, three brain vesicles that develop at the cranial end of the neural tube differentiate to form the prosencephalon, mesencephalon, and rhombencephalon at CS13[11]. Moreover, the brain does not reach definitive organization until after CS15, with the emergence of the telencephalon. Application of sonoembryology to the embryonic period is essential for our comprehension of brain development. Moreover, determining an appropriate developmental stage in which sonography can be applied is important for precise diagnostics[12]. However, exact staging is still difficult because it requires imaging internal structures of the brain at the microscopic level[13].

Morphometrical studies (both two- and three-dimensional) can be analyzed using serial histological sections and visualized via 3D modeling and illustrations[13-15]. However, these methods are laborious and inaccurate. Moreover, histological sections contain artefacts due to the use of fixative, which can cause tissue shrinkage and deformation from dehydration[16]. Finally, very

few morphometric studies of the human embryonic brain have employed the CS[14,15].

Recently, we analyzed morphogenetic and volumetric measurements of the embryonic brain via 3D reconstructions of MR microscopic data[10], and observed dramatic growth at each CS. Thus, we used the same materials in our current study to measure linear segments and specific areas in MR images, similar to what has been reported in previous studies[14,15]. One advantage of this approach is that we were able to accurately measure a suitable plane, allowing for the estimation of a correlation between linear and volumetric measurements (region-by-region) using staged embryos as early as CS13, which corresponds to 6weeks' gestation.

Materials and methods

Embryonic specimens

Approximately 44000 human embryos (comprising the Kyoto Collection) are stored at the Congenital Anomaly Research Center at Kyoto

University[17,18]. In most cases, the pregnancies from which these embryos were derived were terminated during the first trimester for socioeconomic reasons under the Maternity Protection Law of Japan. From this collection, we measured, examined, and staged embryos using criteria provided by O'Rahilly and Müller[11]. Approximately 1200 well-preserved human embryos were judged to be normal by two of the authors (C.U. and S.Y.) based on a gross examination. These embryos were later subjected to MR microscopic imaging; the conditions for which have been previously described elsewhere[17-19].

In the present study, 101 samples from the CS13–CS23 range (nine or 10 samples for each stage, with the exception of CS13, for which there were five samples), and their associated morphometric analyses, were selected from the 1200 MR imaging datasets. The selected embryos were identical to those used in a previous study[10].

Morphometric analysis

MR imaging datasets for each embryo were initially obtained as 256 ×

256 × 512 voxel data[19]. The midsagittal and transverse planes were used according to previous studies, with minor modifications[14,15] (Figure 1). The planes for length and area measurements were digitally resectioned using OsiriX™ software (ver. 4.0, Pixmeo SARL, Geneva, Switzerland).

The following seven segments were measured, which were consistent with a previous study[14]: fronto-occipital diameters (FOa and FOb) and bitemporal diameter (BT) as substitutions for the prosencephalon, mesencephalon length (ML) and mesencephalon height (MH) as substitutions for the mesencephalon, and cerebellar length (CL) and cerebellar height (CH) as substitutions for the rhombencephalon and cerebellum, respectively (Figure 1A). Area measurements were obtained for three regions in the midsagittal section that corresponded to the prosencephalon, mesencephalon, and rhombencephalon[15].

Brain volume was measured by manual segmentation, as described previously[10]. Briefly, brains and ventricles were segmented for 3D reconstruction using the FSL view of the FMRIB Software Library™ (ver. 4.1.9, Analysis Group,

150 FMRIB, Oxford, UK). Three-dimensional brain morphology was computationally
151 reconstructed with Amira™ software (ver. 5.4.0, Visage Imaging, Berlin,
152 Germany).

153 Brain and whole embryo volumes were calculated using OsiriX™
154 software (ver. 4.0, Pixmeo SARL, Geneva, Switzerland). Vesicles were divided
155 into three regions according to the following anatomical landmarks: the
156 supramammillary recess and posterior commissure were used to define the
157 prosencephalon and mesencephalon; the isthmic recess and the isthmic groove
158 were used to define the mesencephalon and rhombencephalon; and the C1
159 vertebral level was used to define the separation between the rhombencephalon
160 and spinal cord[10]. After dimensional matching, a formula was derived to estimate
161 brain volume from the linear measurements. We then analyzed both total brain
162 volume (with the ventricles) and brain volume without the ventricles, since an
163 increase in ventricular volume – a feature of the embryonic period – could have
164 influenced our measurements[5,6,10,15]. The Ethics Committee of the Kyoto
165 University Graduate School and Faculty of Medicine (E986) approved this study.

Results

Linear, area, and volume measurements

1. Linear measurements

The seven length segments evaluated here were linearly correlated with CRL (Table 1, Supplementary figure 1). The correlation coefficient was large for all segments ($r \geq 0.91$), and the slopes of the regression lines were consistent with those of a previous study (with the exception of the CL)[14]. In that prior study, the correlation coefficients for CL and CH were small ($r = 0.14$ for CL and 0.52 for CH) [14].

The seven length segments were plotted by CS group (Figure 2). BT and MH exhibited a nearly linear increase, while other segments increased non-monotonically during CS13 and CS23. A plateau phase between CS19 and CS20 was observed in several segments (FOa, FOb, ML, and CL). It should be noted that we were unable to compare our data with that of a previous study because the segment and CS correlations in that study were not precisely

182 analyzed[14].

183

184 **2. Area measurements**

185 Areas in the midsagittal section that corresponded to the whole brain, as
186 well as the prosencephalon, mesencephalon, and rhombencephalon, were
187 measured (Figure 3). Each area increased between CS13 and CS23.
188 Exponential curves demonstrated a good fit to the data (R^2 = 0.92–0.97), except
189 for the rhombencephalon area (R^2 = 0.85); this finding was consistent with that of
190 a previous study (Table 2)[15].

191

192 **3. Volume measurements**

193 When brain volume was measured without the ventricles (i.e., brain tissue
194 only), we determined that it increased exponentially until CS23. On the other
195 hand, when ventricular volume was included in the measurement, we
196 determined that brain volume increased and then plateaued between CS19 and
197 CS20 (Table 3).

Tissue volumes from the three brain vesicles grew exponentially, though the growth rates differed. Volumes including cavities did not grow exponentially. Rhombencephalon volume increased with a local maximum at CS19, which was affected by cavity growth; namely, the rhombencephalon brain cavity, which becomes the fourth ventricle, reached its maximum volume at CS19.

Large brain cavities, with non-exponential volume changes, are a prominent feature during the early embryonic period. Whole-brain cavity volumes were greater than whole-brain tissue volumes between CS14 and CS18 (Table 3). The ratio of whole-brain cavity to tissue volume reached a maximum at CS17 (ratio = 1.46), after which brain tissue volume became greater than brain cavity volume. The maximum brain cavity to brain tissue volume ratio was noted at CS17 in all three regions and was particularly prominent in the rhombencephalon (prosencephalon: 1.11, mesencephalon: 1.04, and rhombencephalon: 1.84).

Predicting brain volume from linear data and measurements

A formula was generated to estimate whole-brain volume both with and without ventricles, based on matched dimensions. These results are summarized in Table 4. A large correlation was observed with the linear BT measurement (total volume: r = 0.97, non-ventricular volume: r = 0.98). BT was also highly correlated with prosencephalon volume (total volume: r = 0.98, non-ventricular volume: r = 0.99). These correlations were larger than for any other measurement or combination. Other measurements, such as FOb and combinations (e.g., FOa*FOb*BT), were highly correlated with prosencephalon volume, both with and without inclusion of ventricular volume.

For the mesencephalon, all linear measurements examined (ML and MH) were highly correlated with mesencephalon volume, both with and without inclusion of ventricular volume. Correlations were also high for estimating the rhombencephalon (r = 0.80 for CH^3, and 0.92 for CL^3).

Discussion

229 The human embryonic brain develops in a complicated manner over a
230 short period[10], with growth speed and increases in measurements varying
231 according to developmental stage. Thus, the determination of developmental
232 stage is necessary for a more precise detection of embryonic abnormalities.
233 Improvements in sonographic resolution may contribute to our ability to observe
234 detailed and precise embryonic morphology. Nevertheless, it remains unclear
235 how measurements, which reflect developmental features, relate to
236 developmental stages. Classic embryology using histological techniques has
237 revealed morphological features according to developmental stage, and this
238 forms the current basis of sonoembryology. However, classic embryology has
239 provided relatively little morphometric data[14.15]. The present study improved
240 upon previous methods by providing a suitable plane for a morphometric
241 analysis from staged human embryos using MR microscopic data.

242 In the current study, the increases in brain cavity and whole body
243 volume (in relation to the CRL) were comparable to those reported by Blaas'
244 sonoembryologic study[20] (Figure 4). Regarding the prosencephalon, the volume

245 in both studies increased exponentially with overlapping values (Figure 4B). The
246 volume of the mesencephalon was variable across samples, especially for larger
247 specimens (CRL > 20 mm), and increased in a broadly linear manner. With
248 respect to the rhombencephalon, the variance was considerable among smaller
249 samples (CRL > 10 mm). The increasing growth rates of the mesencephalon
250 and rhombencephalon were essentially similar between our study and that of
251 Blaas for samples with CRL's between 10 and 30 mm. Our observations that
252 volume data in the present study were substantially similar to Blaas'
253 sonoembryologic data indicate that our present data are potentially useful.

254 It is known that almost all measurements increase during the
255 embryonic period; thus, linear measurements correlate both with each other and
256 with age. In the current study, all parameters correlated with CRL (Table 1,
257 Supplemental Figure 1). However, this basic analysis does not consider
258 stage-specific features, which are important for precise diagnostics. When we
259 analyzed the relationship of the parameters with CS, several measurements
260 showed non-monotonic increases (i.e., the relationship plateaued, with a local

maximum observed c. CS19−20). Rousian et al.[8] measured the relationship of numerous parameters with CS using 3D sonography and found that brain cavity volume increased with the quadratic function of the CS. The mean brain cavity volume in our study was comparable to that reported by Rousian et al. from CS13 to 17, but not with the quadratic function they used after CS 18 (Figure 5). Analyzing why such discrepancies arise may provide clues for further understanding stage-specific morphological features during development.

Linear measurements, which can also be used to estimate volume, may be preferable to other measurements because they are related to both brain development and growth. Thus, BT is a good marker for estimating human embryonic brain development and growth, as has been mentioned in previous studies[1,4,7,13, 21]. The present study included embryos at earlier stages (between CS13 and CS15) in which the telencephalon was not prominent; thus, our results demonstrated that linear measurement values, such as BT and volume, are useful between CS13 and CS15.

It is difficult to compare our linear measurements with most previous

277 sonographic measurements[3,5,6,7], as the measurements used in the present
278 study were selected from classical embryonic studies[14,15]. Thus, with the
279 exception of MH and ML, most of the linear measurement definitions we
280 employed differed from those of recent studies[5,9]. Using sonography, Tanaka et
281 al.[9] presented length measurements (including ML and MH) at every day of
282 gestation between days 49 and 69. The length of ML in our study was shorter
283 than that of the Tanaka at all stages between CS 15 and 23. The MH length in
284 our study was also shorter than that of Tanaka et al. between CS 15 and 20, but
285 larger after CS 21 (data not shown). Note that the comparison may not be
286 entirely reliable as we compared the ML and MH data using the predicted CS
287 from the day of gestation[13]. Selection and reevaluation of linear measurements
288 that allow comparisons of data among sonoembryonic studies will be necessary
289 for future studies. Further, the relationship between CS and embryo age is a
290 critical issue to consider. Embryological studies demonstrate that considerable
291 variability is observed in the size and developmental stage among human
292 embryos at a given gestational age[22]. Thus, the embryonic age that is used in

clinics cannot be used to determine the developmental stage (CS), as prenatal development may not proceed at the same speed in every embryo.

The present study provided a morphometric analysis from staged human embryos using MR microscopic data. Morphometric data, according to human embryonic stages, are valuable for correcting and comparing sonographic data. The present approach may contribute to improvements in prenatal diagnostics by enabling the selection of more suitable measurements during earlier embryonic stages.

References

1 Blaas HG. Detection of structural abnormalities in the first trimester using ultrasound. Best Pract Res Clin Obstet Gynaecol 2014;28:341–53.

2 Pooh RK, Shiota K, Kurjak A. Imaging of the human embryo with magnetic resonance imaging microscopy and high-resolution transvaginal 3-dimensional sonography: human embryology in the 21st century. Am J Obste Gynecol 2011;204:77.e1-16.

3. Blaas HG, Eik-Nes SH, Berg S, *et al*. In-vivo three-dimensional ultrasound reconstructions of embryos and early fetuses. Lancet 1998;352:1182–6.

4. Blaas HG, Eik-Nes SH, Bremnes JB. Embryonic growth. A longitudinal biometric ultrasound study. Ultrasound Obstet Gynecol 1998;12:346–54.

5. Blaas HG, Eik-Nes SH, Kiserud T, *et al*. Early development of the forebrain and midbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. Ultrasound Obstet Gynecol 1994;4:183–92.

6. Blaas HG, Eik-Nes SH, Kiserud T, *et al*. Early development of the hindbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. Ultrasound Obstet Gynecol 1995;5:151–60.

7. Gijtenbeek M, Bogers H, Groenenberg IAL, *et al*. First trimester size charts of embryonic brain structures. Hum Reprod 2014;29:201–7.

8. Rousian M, Hop WC, Koning AHJ, *et al*. First trimester brain ventricle fluid and embryonic volumes measured by three-dimensional ultrasound with the use of I-Space virtual reality. Hum Reprod 2013;28:1181–9.

9. Tanaka H, Senoh D, Hata T. Intrauterine sonographic measurement of

325 embryonic brain vesicle. Hum Reprod 2000;15:1407–12.

326 10 Shiraishi N, Katayama A, Nakashima T, *et al*. Morphology and morphometry

327 of the human embryonic brain: A three-dimensional analysis, NeuroImage

328 2015;115:96–103.

329 11 O'Rahilly R, Müller F. Developmental stages in human embryos: including a

330 revision of Streeter's Horizons and a survey of the Carnegie Collection.

331 Washington, D.C.: Carnegie Institution of Washington, 1987.

332 12. Verwoerd-Dikkeboom CM, Koning AH, van der Spek PJ, *et al*. Embryonic

333 staging using a 3D virtual reality system. Hum Reprod 2008;23:1479–84.

334 13. O'Rahilly R, Müller F. Developmental stages in human embryos: revised and

335 new measurements. Cells Tissues Organs 2010;192:73–84.

336 14 Desmond ME, O'Rahilly R. The growth of the human brain during the

337 embryonic period proper. 1. Linear axes. Anat Embryol (Berl) 1981;162:137–51.

338 15 Levitan ML, Desmond ME. Expansion of the human embryonic brain during

339 rapid growth: area analysis. Anat Rec 2009;292:472–80.

340 16. van Duijn S, Nabuurs RJ, van Rooden S, *et al*. MRI artifacts in human brain

341 tissue after prolonged formalin storage. Magn Reson Med 2011;65:1750–8.

342 17 Nishimura H, Takano K, Tanimura T, *et al*. Normal and abnormal

343 development of human embryos: first report of the analysis of 1,213 intact

344 embryos. Teratology 1968;1:281–90.

345 18 Shiota K, Yamada S, Nakatsu-Komatsu T*, et al*. Visualization of human

346 prenatal development by magnetic resonance imaging (MRI). Am J Med Genet

347 A 2007;143A:3121–6.

348 19 Matsuda Y, Ono S, Otake Y, *et al*. Imaging of a large collection of human

349 embryo using a super-parallel MR microscope. Magn Reson Med Sci

350 2007;6:139–46.

351 20. Blaas H-G. The embryonic examination. Ultrasound studies on the

352 development of the human embryo. Thesis, Norwegian University of Science

353 and Technology, Trondheim, pp 75–76, 1999.

354 21. Rabelink IAA, Degen JEM, Kessels MEW, *et al*. Variation in early growth.

355 Eur J Obstet Gynecol Reprod Biol 1994;53: 39–43.

356 22. Shiota K. Variability in human embryonic development and its implications

for the susceptibility to environmental teratogenesis. Birth Defects Res A 2009;85:661–6.

Figure legends

Figure 1. MR images of midsagittal and transverse sections

(A-i) A human brain at CS23. The plane corresponding to the transverse section is indicated by a red dashed line on the midsagittal section, while the plane corresponding to the midsagittal section is indicated by a red dashed line on the transverse section. The plane was shifted from the “true” midsagittal section (indicated by a blue dashed line) because the “true” line does not include the telencephalon.

FOa: fronto-occipital diameter (primordium chiasmatis), FOb: fronto-occipital diameter (velum transversum), ML: mesencephalic length, MH: mesencephalic height, CL: cerebellar length; CH: cerebellar height, BT: bitemporal diameter (i.e., the distance between the lateral surface of the right and left temporal prominences).

(A-ii) Boundaries between the three primary brain vesicles were established by drawing a perpendicular line connecting the dorsal and ventral primary fissures,

separating the prosencephalon from the mesencephalon and the mesencephalon from the rhombencephalon.

Pr: prosencephalon, Me: mesencephalon, Rh: rhombencephalon, Md: sum of Pr, Me, and Rh.

Measurements were obtained from two previous studies (Desmond and O'Rahilly, 1981; Levitan and Desmond, 2009).

(B) Human brain at CS16.

(C) Human brain at CS13.

.

Figure 2. Embryonic brain length measurements according to Carnegie stages.

Figure 3. Area measurements between CS13 and CS23.

The rhombencephalon in our data is compared with "whole cerebellum" defined in the previous study[15].

Figure 4. Comparison of the present volume measurements with Blaas' study.

(A) Increase in brain cavity and whole body volume in relation to the CRL.

(B) Prosencephalon, mesencephalon, and rhombencephalon volumes in relation to the CRL.

Blue: the present study, red: data from Blaas' study (pp 76 in Ref. 20). The prosencephalon volume was calculated as the sum of the hemispheres and the diencephalon.

Figure 5. Brain cavity volume in relation to the CS.

The present volume measurements were compared to those reported by Rousian et al.

The mean volume of our data overlapped, from CS13 to 17, with the quadratic function employed by Rousian et al[8].

Supplementary Figure 1. Correlation between the seven length segments measured and the CRL. The data imply that brain size measurements are useful indicators of linear embryonic neural development.

Table 1. Correlation between CR-length and measured segments

Segment (Ymm)	Present study				Desmond & O'Rahilly (1980)			
=a×[CRL] + b	a	b	n	r	a	b	n	r
FOa	0.21	0.64	101	0.93	0.25	-0.57	83	0.92
FOb	0.28	0.36	101	0.95				
BT	0.34	-0.68	101	0.96	0.27	-0.78	75	0.97
ML	0.16	0.77	101	0.94	0.17	0.32	83	0.90
MH	0.14	-0.01	101	0.96	0.12	-0.29	85	0.92
CL	0.07	0.17	101	0.95	0.02	0.80	74	0.14
CH	0.06	-0.22	101	0.91	0.07	-0.06	77	0.52

CRL; Crown-Rump length, r; correlation coefficient

Table 2. Correlation between Carnegie stages and measured areas

Area (Y mm^2)	Present study				Levitan & Desmond (2009)			
$= a \times e^{b[CS]}$	$a \times 10^{-2}$	$b \times 10^{-1}$	n	R^2	$a \times 10^{-2}$	$b \times 10^{-1}$	n	R^2
Median section	6	3.11	101	0.92	3.33	4.37	52	0.96
Prosencephalon	0.6	3.88	101	0.96	0.74	4.94	58	0.95
Mesencephalon	0.8	3.26	101	0.95	2.92	3.84	58	0.91
Rhombencephalon *	11	2.35	101	0.85	0.03	4.95	52	0.84

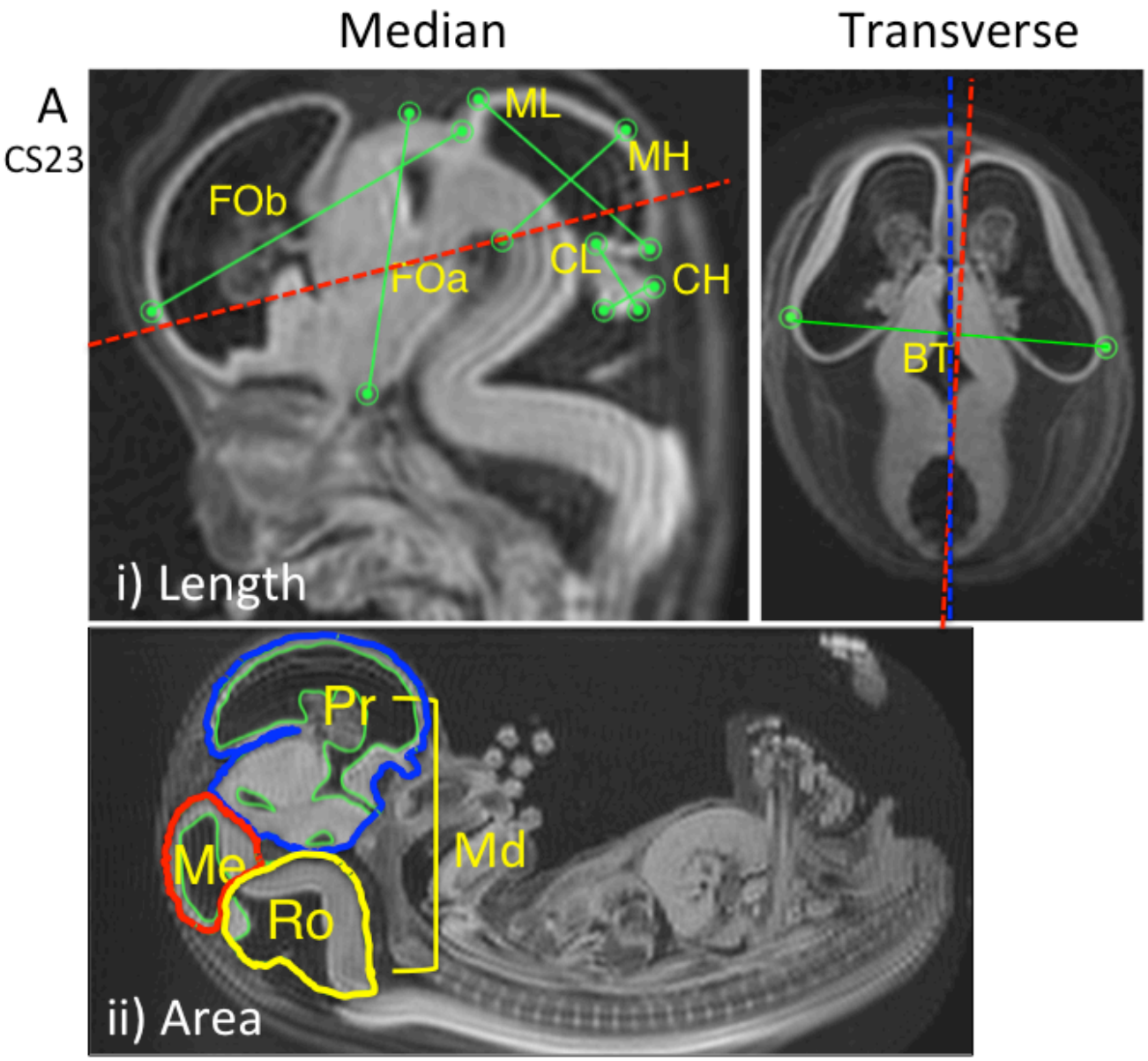
CS = Carnegie stages; *; whole cerebellum in Levitan & Desmond (2009)

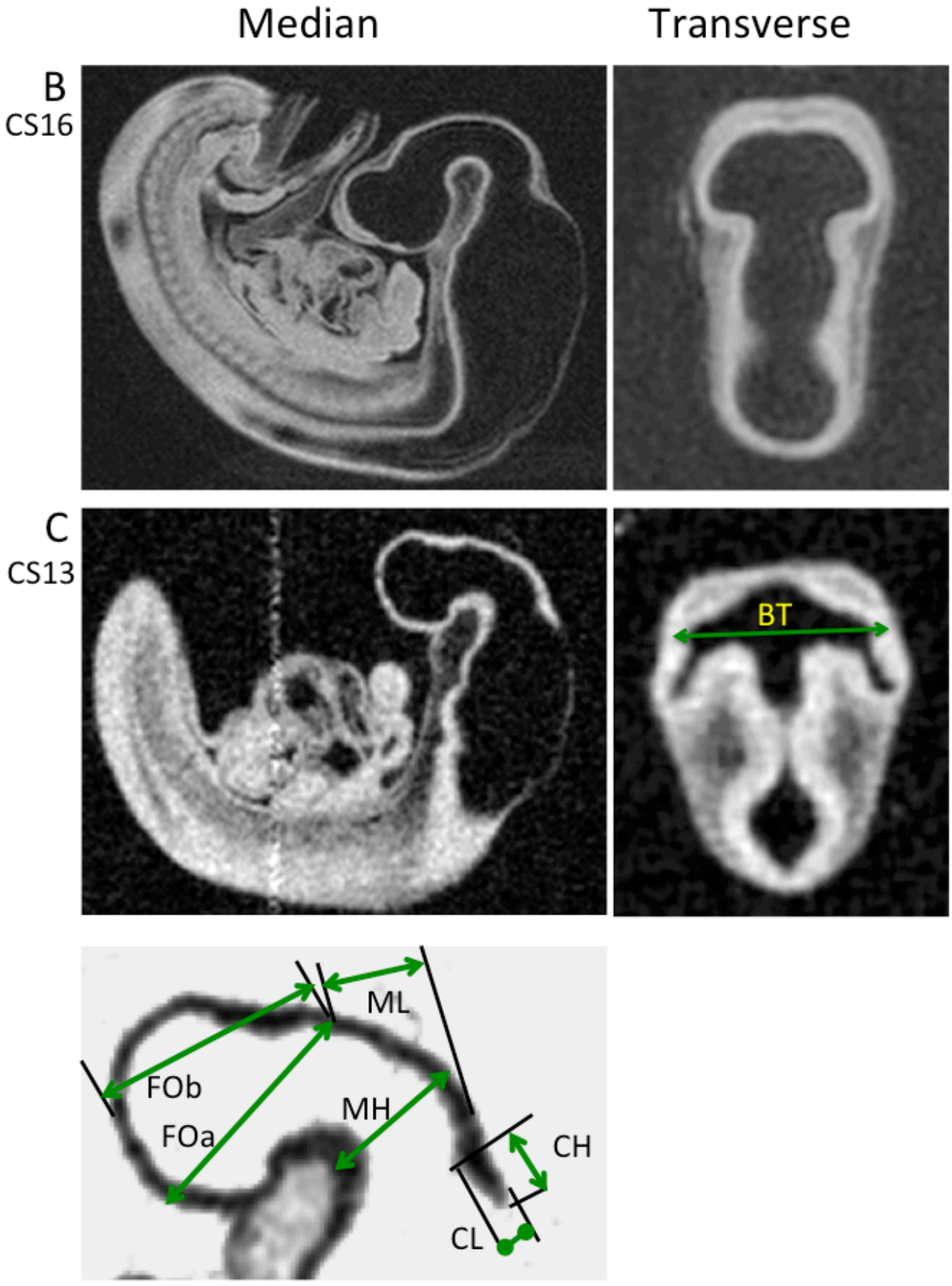
Table 3. Brain volume of human embryos measured by manual segmentation

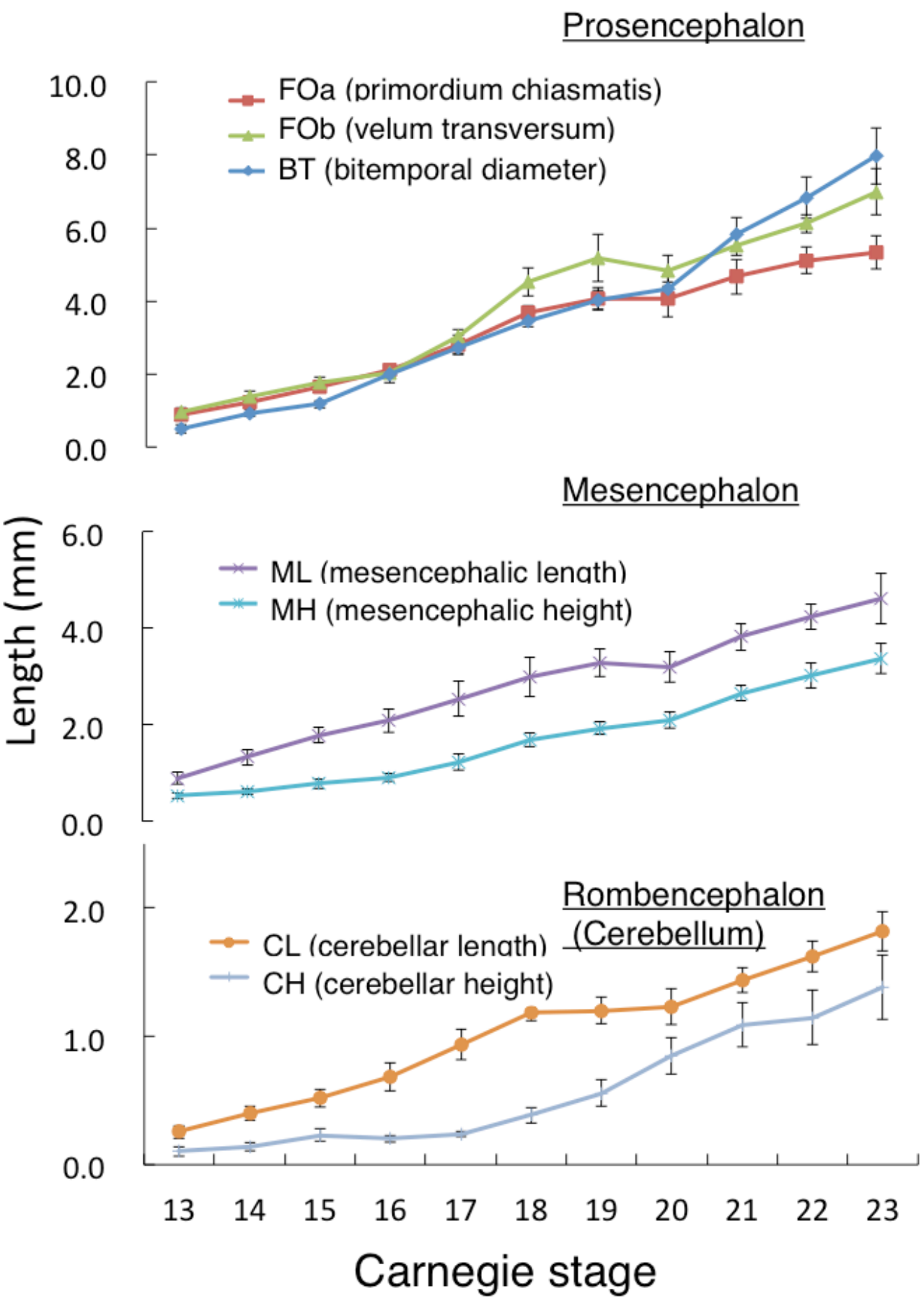
Carnegie		Whole brain					Prosencephalon					Mesencephalon					Rhombencephalon				
		mean volume (mm^3)					mean volume (mm^3)					mean volume (mm^3)					mean volume (mm^3)				
stage	n	total	(SD)	tissue	cavity	ratio	total	(SD)	tissue	cavity	ratio	total	(SD)	tissue	cavity	ratio	total	(SD)	tissue	cavity	ratio
13	5	2.1	0.7	1.2	0.9	0.82	0.5	0.2	0.3	0.2	0.77	0.3	0.1	0.2	0.1	0.40	1.3	0.4	0.7	0.7	0.96
14	9	5.7	1.4	2.6	3.1	1.16	1.2	0.4	0.7	0.6	0.88	0.7	0.1	0.4	0.3	0.60	3.8	0.9	1.6	2.3	1.43
15	9	11.4	1.6	5.5	6.0	1.10	2.6	0.4	1.5	1.2	0.79	1.3	0.2	0.8	0.5	0.67	7.5	1.2	3.2	4.3	1.34
16	10	16.3	2.6	7.4	8.9	1.20	4.1	1.1	2.1	2.0	0.96	2.0	0.4	1.2	0.9	0.74	10.1	1.3	4.2	6.0	1.45
17	10	29.9	6.2	12.2	17.8	1.46	9.0	2.5	4.3	4.7	1.11	3.9	1.0	1.9	2.0	1.04	17.1	3.0	6.0	11.1	1.84
18	9	57.7	7.5	27.6	30.1	1.09	19.3	2.7	9.8	9.6	0.98	8.1	0.9	4.1	4.0	0.98	30.3	4.8	13.7	16.6	1.21
19	10	79.0	8.9	43.9	35.1	0.80	29.9	4.6	17.1	12.8	0.75	11.6	1.2	6.6	4.9	0.74	37.5	4.9	20.1	17.4	0.87
20	10	81.9	19.0	52.8	29.1	0.55	36.8	8.2	23.0	13.8	0.60	11.3	2.6	6.8	4.5	0.66	33.8	9.0	22.9	10.8	0.47
21	10	145.1	16.6	95.3	49.8	0.52	74.0	14.5	47.5	26.5	0.56	19.0	2.8	12.5	6.5	0.52	52.1	6.2	35.3	16.9	0.48
22	9	206.4	39.0	135.2	71.1	0.53	115.5	29.0	70.5	45.0	0.64	25.7	4.1	17.9	7.8	0.44	65.2	10.2	46.8	18.3	0.39
23	10	286.3	61.7	189.1	97.2	0.51	184.5	50.5	111.0	73.5	0.66	28.9	4.0	21.9	7.0	0.32	73.1	10.0	56.3	16.7	0.30

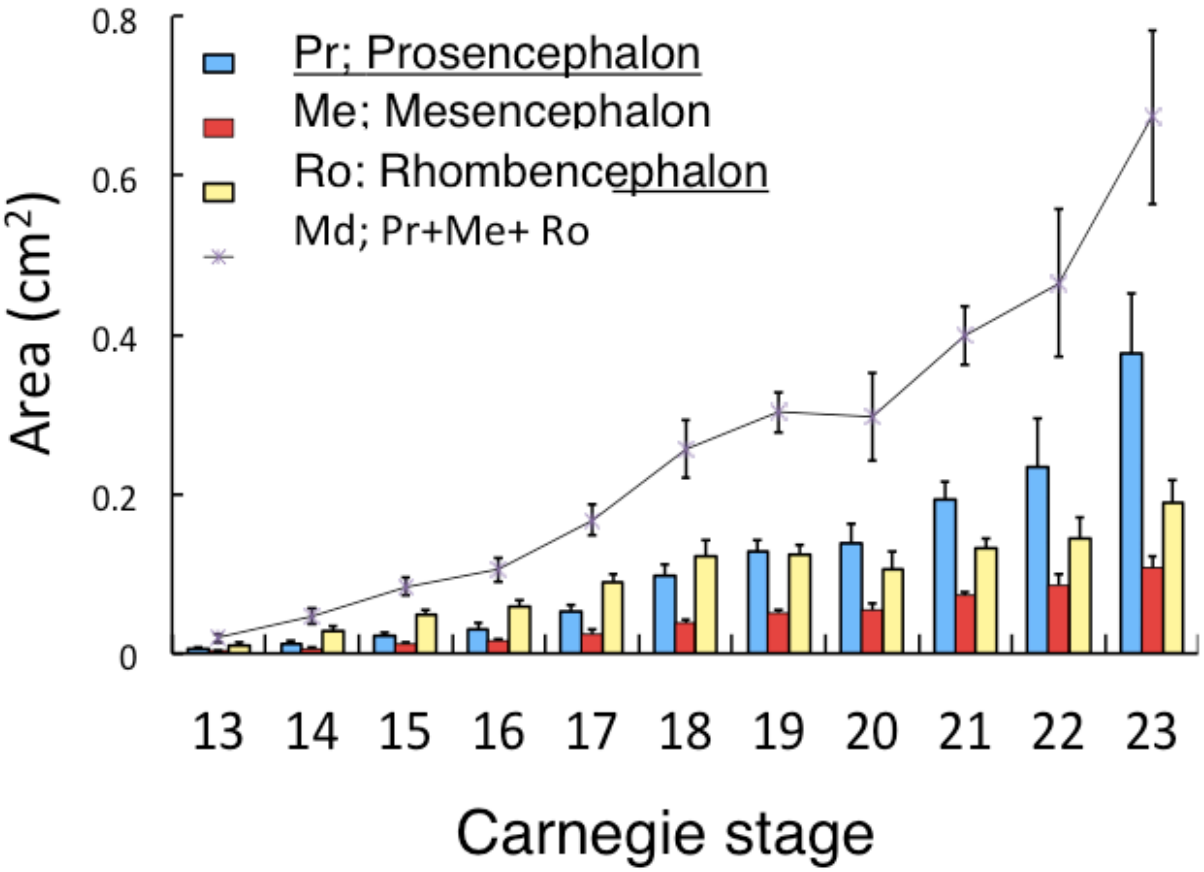
Table 4. Calculation of brain volume from length measurements

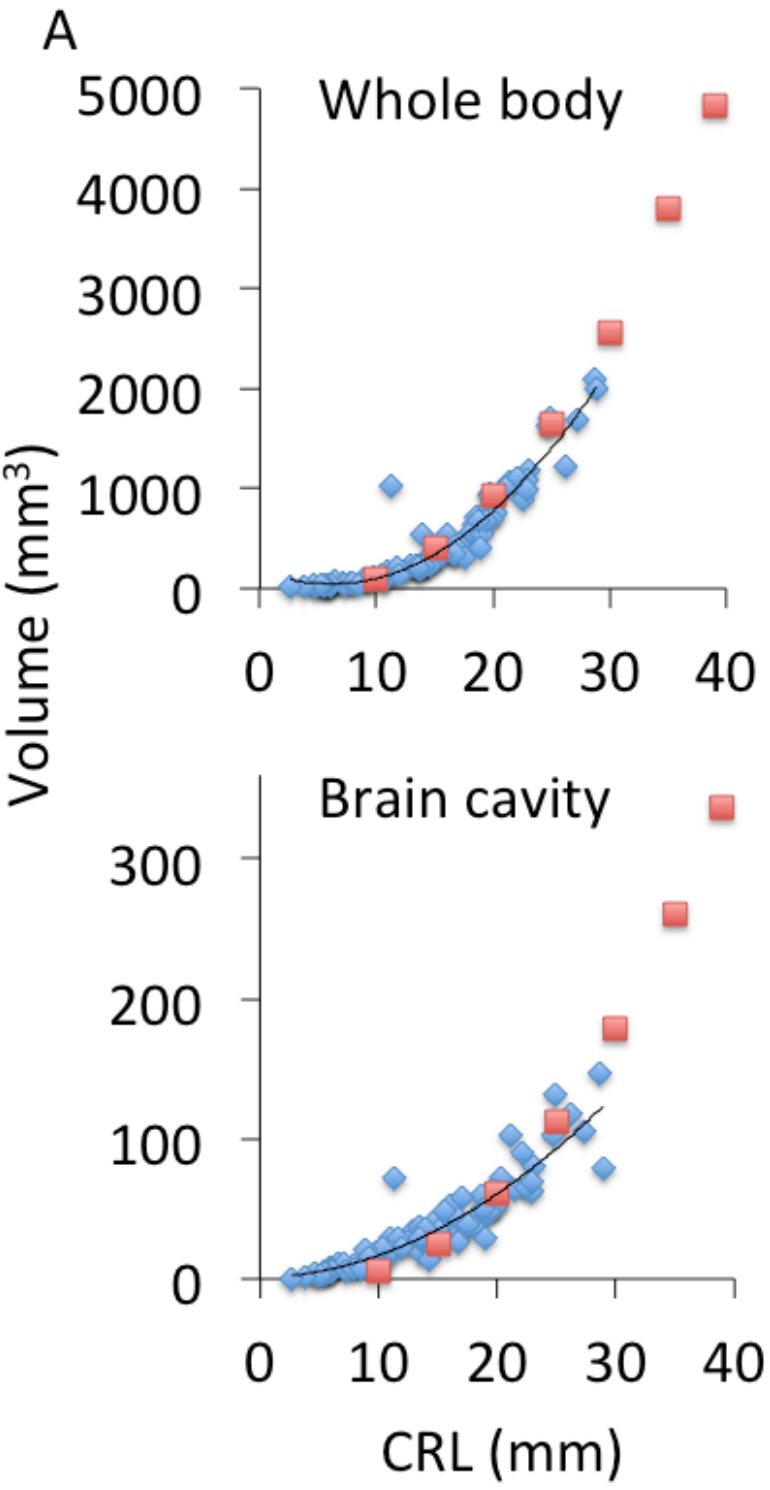
Formula $V\ (mm^3) = a\ [X][Y][Z]+b$	a	[X][Y][Z]	+b	r
Volume with ventricles				
Whole brain	0.52	$[BT]^3$	26.2	0.97
Prosencephalon	0.92	$[FOa]^3$	-9.13	0.86
	0.49	$[FOb]^3$	-8.21	0.93
	0.34	$[BT]^3$	4.05	0.98
	0.59	[FOa][FOb][BT]	-7.50	0.97
Mesencephalon	0.70	$[MH]^3$	3.27	0.94
	0.28	$[ML]^3$	0.90	0.95
Rhombencephalon	18.1	$[CH]^3$	19.8	0.80
	11.2	$[CL]^3$	9.87	0.92
Volume without ventricles				
Whole brain	0.35	$[BT]^3$	12.7	0.98
Prosencephalon	0.57	$[FOa]^3$	-6.39	0.88
	0.30	$[FOb]^3$	5.18	0.94
	0.21	$[BT]^3$	2.11	0.99
	0.36	[FOa][FOb][BT]	-5.03	0.97
Mesencephalon	0.53	$[MH]^3$	1.42	0.96
	0.21	$[ML]^3$	-0.21	0.95
Rhombencephalon	15.1	$[CH]^3$	10.3	0.85
	8.99	$[CL]^3$	2.68	0.94

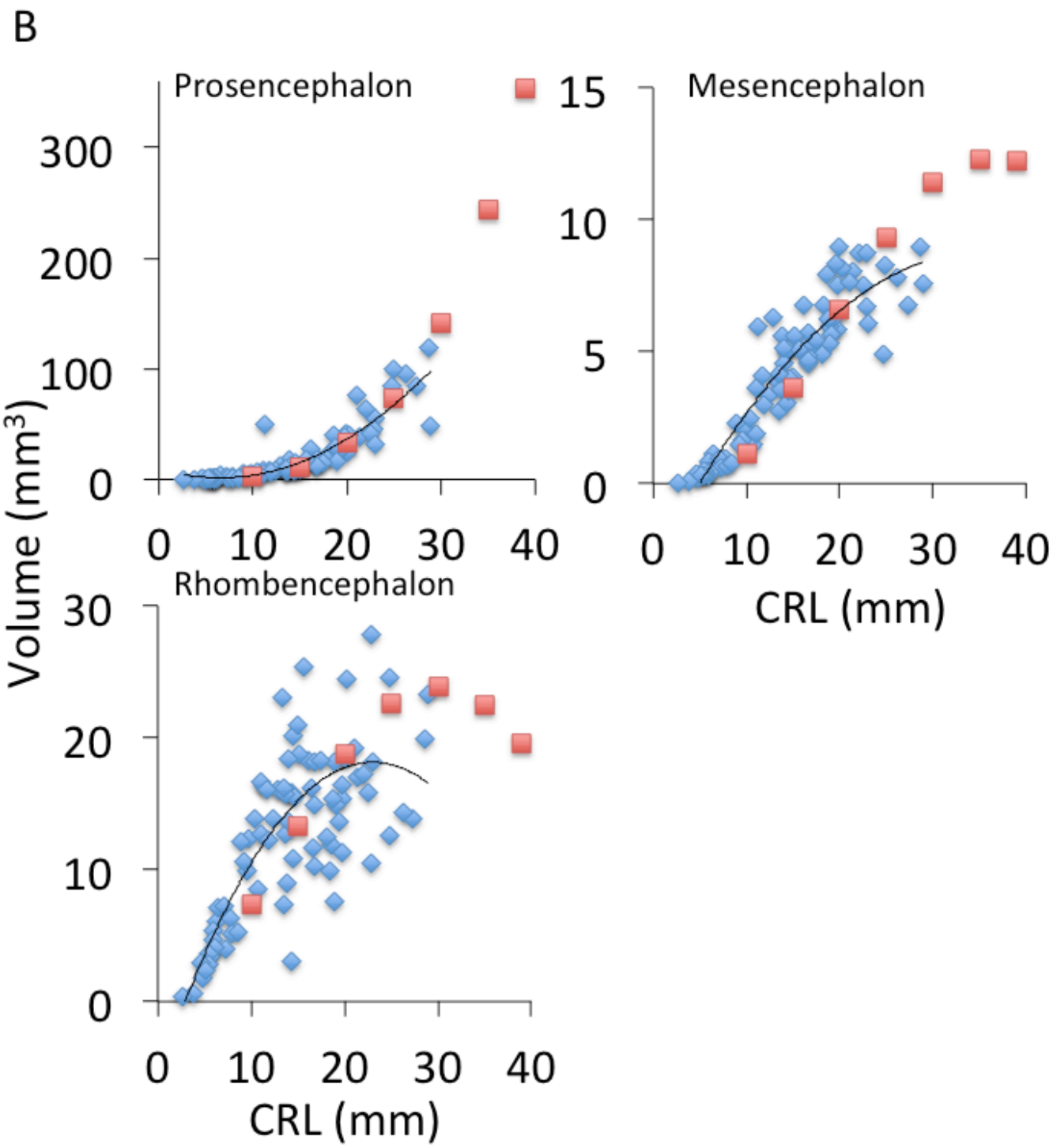

Median
Transverse
A
CS23
ML
MH
FOb
FOa
CL
CH
BT
i) Length
Pr
Md
Me
Ro
ii) Area

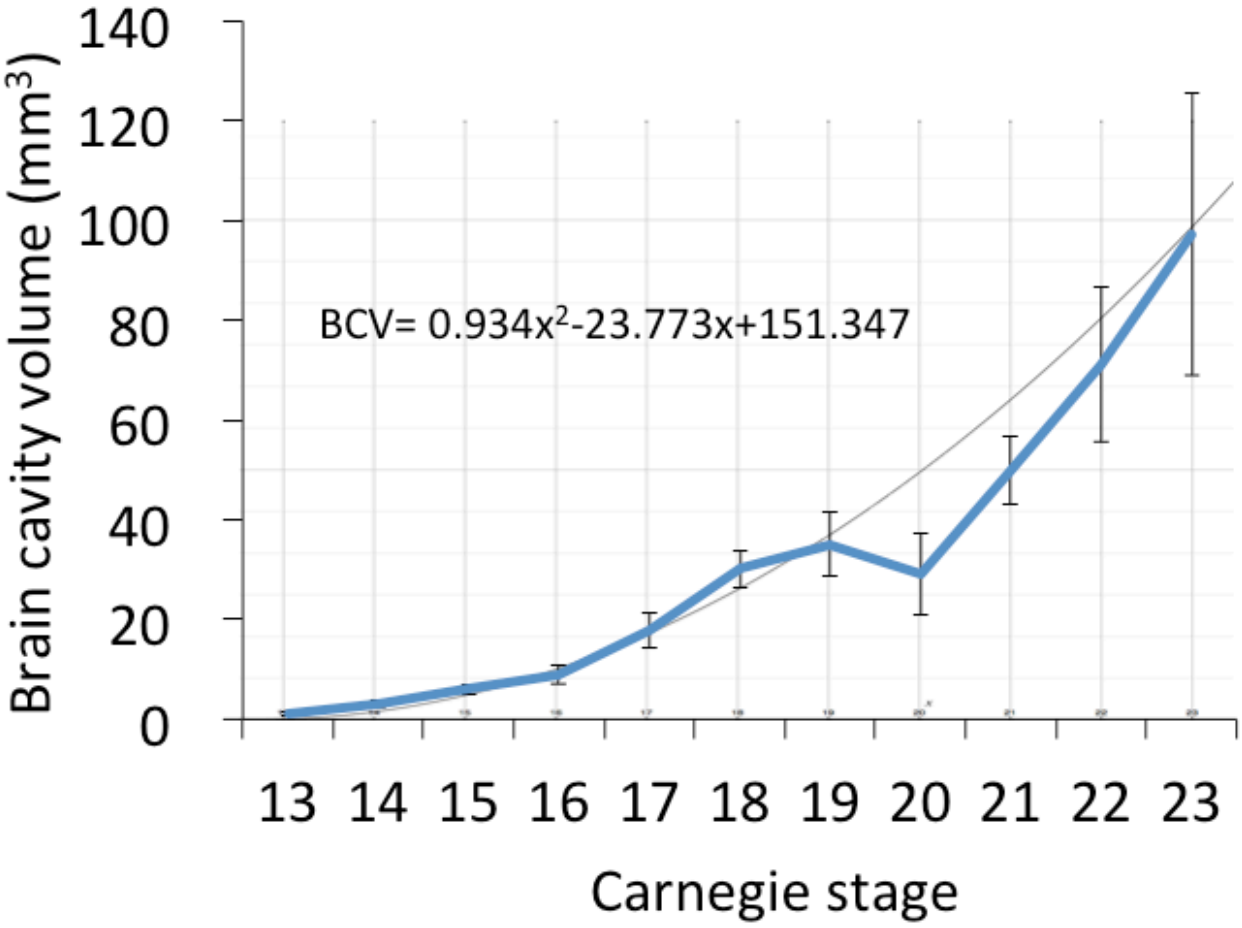

Median
Transverse
B
CS16
C
CS13
BT
ML
FOb
FOa
MH
CH
CL


Prosencephalon
FOa (primordium chiasmatis)
FOb (velum transversum)
BT (bitemporal diameter)
10.0
8.0
6.0
4.0
2.0
0.0
Mesencephalon
ML (mesencephalic length)
MH (mesencephalic height)
6.0
4.0
2.0
0.0
Rombencephalon
(Cerebellum)
CL (cerebellar length)
CH (cerebellar height)
2.0
1.0
0.0
Length (mm)
13
14
15
16
17
18
19
20
21
22
23
Carnegie stage


0.8
0.6
0.4
0.2
0
Area (cm²)
Pr; Prosencephalon
Me; Mesencephalon
Ro: Rhombencephalon
Md; Pr+Me+ Ro
13
14
15
16
17
18
19
20
21
22
23
Carnegie stage


A
Whole body
5000
4000
3000
2000
1000
0
Volume (mm3)
0
10
20
30
40
Brain cavity
300
200
100
0
0
10
20
30
40
CRL (mm)


B
Prosencephalon
Mesencephalon
Rhombencephalon
Volume (mm³)
CRL (mm)
CRL (mm)
0
100
200
300
0
5
10
15
0
10
20
30
40


Brain cavity volume (mm³)
140
120
100
80
60
40
20
0
BCV= 0.934x²-23.773x+151.347
13 14 15 16 17 18 19 20 21 22 23
Carnegie stage