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COMMUNICATION

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A three-dimensional analysis of the development of cranial nerves in human embryos

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Abstract

To increase our understanding of the etiology of specific neurological disorders (e.g., Duane syndrome, glossoptosis in Pierre Robin sequence), proper knowledge of anatomy and embryology of cranial nerves is necessary. We investigated cranial nerve development, studied histological sections of human embryos, and quantitatively analyzed the 3D reconstructions. A total of 28 sectioned and histologically stained human embryos (Carnegie stage [CS] 10 to 23 [21–60 days of development]) were completely digitalized by manual annotation using Amira software. Two specimens per stage were analyzed. Moreover, quantitative volume measurements were performed to assess relative growth of the cranial nerves. A chronologic overview of the morphologic development of each of the 12 cranial nerves, from neural tube to target organ, was provided. Most cranial nerves start developing at CS 12 to 13 (26–32 days of development) and will reach their target organ in stage 17 to 18 (41–46 days). In comparison to the rest of the developing brain, a trend could be identified in which relative growth of the cranial nerves increases at early stages, peaks at CS 17 and slowly decreases afterwards. The development of cranial nerves in human embryos is presented in a comprehensive 3D fashion. An interactive 3D-PDF is provided to illuminate the development of the cranial nerves in human embryos for educational purposes. This is the first time that volume measurements of cranial nerves in the human embryonic period have been presented.

KEYWORDS
3D, anatomy, cranial nerves, embryology

1 | INTRODUCTION

Knowledge of embryonic development is essential to improve our understanding of the human (adult) anatomy but also to help us

Johannes A. Smit and Karl Jacobs are shared first-authors.

understand the etiology of congenital disorders (e.g., Duane's retraction syndrome, glossoptosis in Pierre Robin sequence, Marcus-Gunn jaw winking ptosis and Möbius syndrome). However, embryology is a complex subject and three-dimensional (3D) insight is of key importance to comprehend the various developmental processes. Nonetheless, educational images often lack a 3D perspective, which hampers full understanding of this difficult topic (de Bakker et al., 2016).

To facilitate 3D insight in human development, the project "*3D Embryo Atlas*" has been established (de Bakker et al., 2016). This atlas allows the human embryo to be studied in a 3D perspective by interactively examining the 3D models. This is relevant, since a significant part of the available literature describing human embryonic development is dated, often based on embryology books and studies from over a 100 years ago, which are mostly based on animal and human embryology data combined and cannot be verified anymore (His, 1895; Keibel & Elze, 1908; Keibel & Mall, 1910; O'Rahilly, 1975).

This manuscript covers detailed descriptions of the development of all 12 cranial nerve pairs (Supplemental Data File I), including quantitative analyses of the cranial nerve volumes throughout development. This description will be illustrated by images of the 3D models and supplementary 3D-PDF file. The 3D-PDF file enables the user to interactively study the development of these structures in subsequent stages of embryology. By visualizing development, both physiological embryogenesis and development of congenital malformations can be better understood.

2 | MATERIALS AND METHODS

Digital images of histological sections of 28 human embryos of the Carnegie collection (Silver Spring, MD, USA), ranging in age from 21 to 60 days of development (Carnegie stages [CS] 10 to 23) were captured (de Bakker et al., 2016). Two specimens per stage were used to study the development of the cranial nerves. Detailed 3D reconstructions were made by manually segmenting each structure in histologically stained sections, using Amira (version 5.3–5.6, Thermo Fisher Scientific, MA, USA). This method was described earlier (de Bakker et al., 2012; de Bakker et al., 2016). The STROBE guideline (Strengthening the reporting of observational studies in epidemiology) was followed for this study.

Each cranial nerve was bilaterally segmented in each embryo as far as they were already present in the particular specimen. The relative inter-observer variability between the research assistants that manually traced the nerves ranged from 0.29% to 1.87% (de Bakker et al., 2016). In the framework of the making of a 3D digital atlas of human development, all other organs and structures were reconstructed as well, permitting reliable positioning relative to other structures. Histologically stained sections of the embryos from the Carnegie collection were imaged, aligned, and segmented. The 3D models were reconstructed in the 3D reconstruction package of Amira. Surfaces were then smoothed (without loss of essential details) via knowledge driven modeling in Blender (version 2.51–2.76, https://www.blender.org). The Blender models were imported into Deep Exploration (version 6.5 CSE, part of Corel DESIGNER Technical Suite X5 http://www.corel.com). After final small corrections in the object tree and material list, the models were exported as Universal 3D (.u3d) file and imported into Adobe Acrobat XI Pro to create an interactive 3D-PDF file, containing all reconstructed cranial nerves and additional structures (e.g., neural tube, eyes, skin) per stage and a user interface to control the visibility of all structures. Data analysis was performed on the segmented sections and the 3D models. The 3D-PDF was added to this manuscript as supplementary data and can be viewed with a recent version of Adobe Reader® (X or higher, Adobe Systems, CA, USA), with JavaScript and 3D content playing enabled.

Amira (version 5.3–5.6) was used to measure the volumes of all reconstructed structures. First, the magnification, or pixel size in X- and Y-direction, of the digitalized histological sections, and the section thickness (Z-value) were determined, to calculate the voxel volume (de Bakker et al., 2016). Volumes of organs and structures were then determined by counting the number of pixels within the manual outline of the structure on a histological section, summing this number for all sections and multiplying by the voxel volume. This method follows the principle of Cavalieri that in a systematic sample of sections, an unbiased estimate of volume can be obtained as the product of summed area times distance (Chieco et al., 2013). To test differences in relative growth between embryos of different ages, Statistical Package for the Social Sciences (SPSS) version 25 (IBM, IL, USA) was used to identify specific growth patterns by using the ANOVA (Analysis of Variance) test. The threshold for statistical significance was set to a p-value <0.05.

2.1 | Standard protocol approvals, registrations, and patients consents

Because of the retrospective nature of this study, using embryos from a historical collection, the ethical approval was waived by the institution's research ethics committee. The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research (Iwanaga et al., 2022).

2.2 | Data availability policy

For the purpose of replicating procedures and results, all qualified investigators can request data after approval from the corresponding author.

3 | RESULTS

Histological sections of 28 human embryos were reconstructed in a 3D perspective. The original sections and the accompanying 3D models were used to study the development of the cranial nerves. A detailed description of the development of the 12 paired cranial

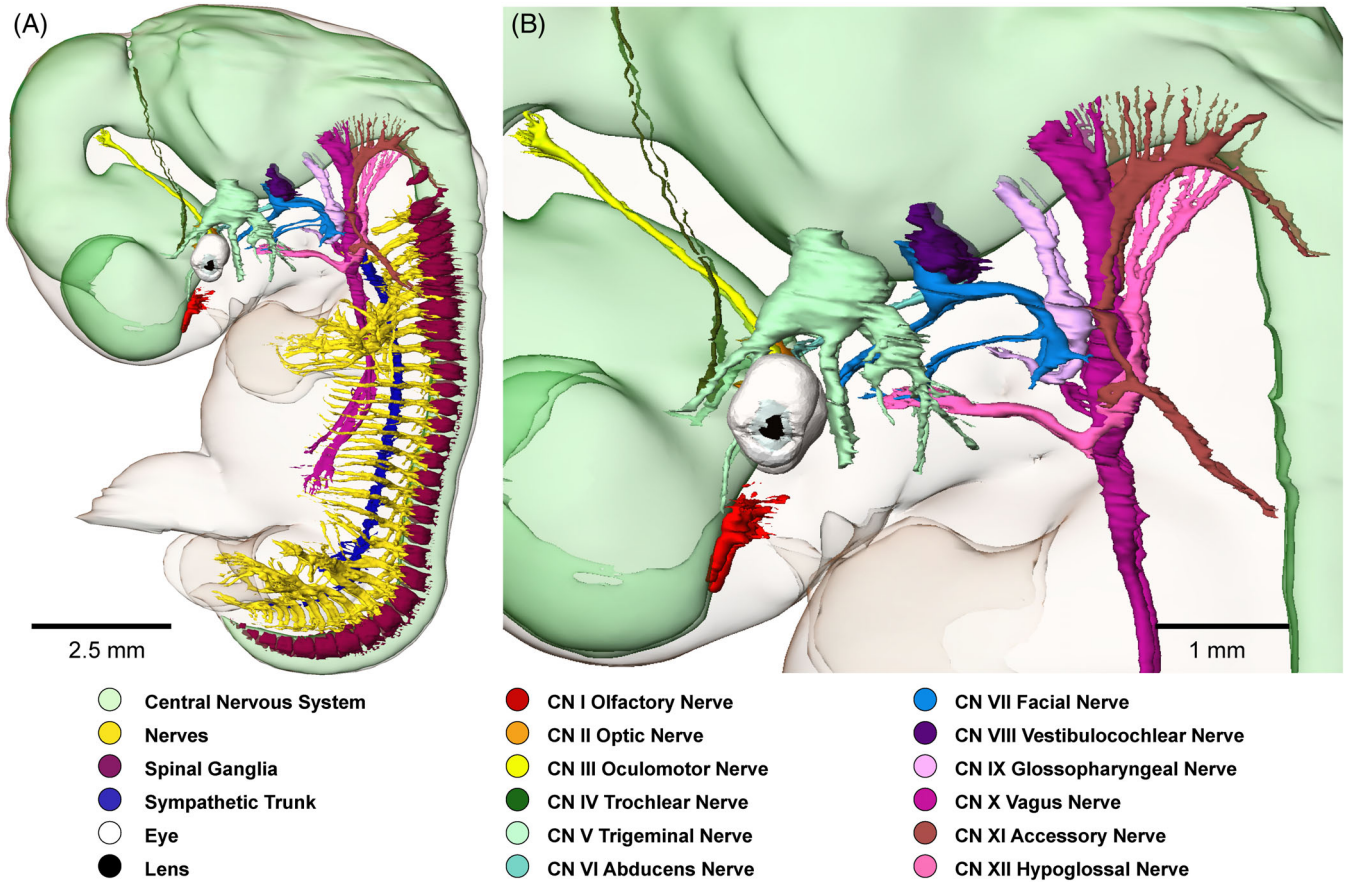

FIGURE 1 3D reconstruction of central and peripheral parts of the nervous system. (A) Lateral view on a 3D reconstruction of the central nervous system, peripheral nerves, spinal ganglia, and sympathetic trunk of a CS 18 specimen #6524 (44–48 days of development) human embryo. (B) Detailed reconstruction of the head region showing the origin of the cranial nerves in relation to the neural tube. CS, Carnegie stage.

nerves will be provided, illustrated with images of the constructed 3D models. Three-dimensional models of CS 13 (28–32 days of development), 16 (37–42 days), 18 (44–48 days), and 21 (53–54 days) are attached as a supplementary 3D-PDF file.

The initial appearance of the cranial nerves has been studied in the reconstructed embryos (Figure 1). Embryos at CS 10 and 11 (21–26 days of development) showed no sign of cranial nerve development, as expected as neurulation is an ongoing process in these embryos. In CS 12 or early CS 13 embryos (resp. 26–30/28–32 days of development), dependent on the studied specimen, the initial development of four cranial nerves (CN II, V, VII, and VIII) can be evidently recognized. Cranial nerve II develops as outgrowth of the neural tube and is therefore immediately connected with its target organ: the optic vesicle. The development of CN IX and X is identifiable only at CS 13. Cranial nerves XI and XII can also be distinguished in CS 13 or CS 14 (31–35 days of development), depending on the individual specimen. Cranial nerves VI and III are found in either a CS 15 or CS 16 (resp. 35–38/37–42 days of development), embryo and CN IV at CS 16 or 17 (42–44 days of development). Finally, the last cranial nerve to develop is CN I, which was found no earlier than at CS 18 (44–48 days of development).

Figure 1 shows a reconstruction of the central and peripheral parts of the nervous system in a human CS 18 embryo (specimen #6524), in which the different parts of the nervous system are displayed: the central nervous system, peripheral nerves, spinal ganglia and sympathetic trunk (Figure 1A). Also, a more detailed figure of the origin of the cranial nerves in relation to the neural tube is shown (Figure 1B). Here, the 12 paired cranial nerves can be distinguished. (Figure 1B).

Figure 2 shows a 3D reconstruction of the cranial nerves in relation to the five brain vesicles in a more developed embryo, CS 23 (specimen #950). The telencephalon gives rise to CN I, the diencephalon to CN II. CN III and IV originate from the mesencephalon and CN V, VI, and VII from the metencephalon. Finally, the remaining five cranial nerves; CN VIII, CN IX, CN X, CN XI, and CN XII are originated from the myelencephalon.

Figure 3 illustrates the stage at which the target organ is reached by the cranial nerves, which is displayed in a more extensive overview in Table 1. This table shows the different branches of the cranial nerves and when they reach their target organs. To illustrate; CN V and its different branches are displayed. A 3D reconstruction of CN V and its main branches in a human CS 23 embryo (specimen #950) is enclosed with this manuscript (Figure 4). The

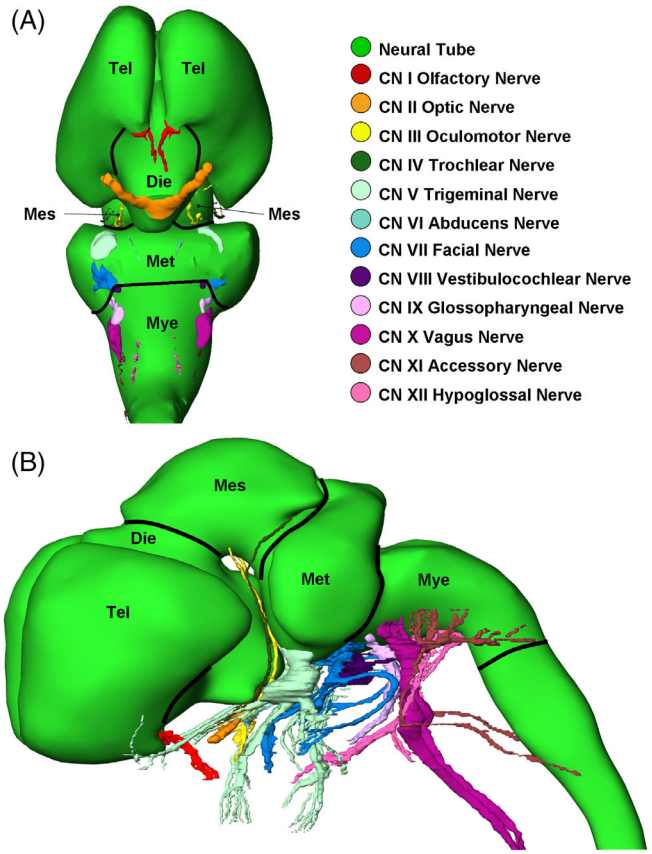

FIGURE 2 Cranial nerves in relation to the brain vesicles. (A) View on the caudal surface of the base of the developing brain in CS 23 specimen #950 (56–60 days of development) including partial reconstruction of the cranial nerves showing their relationship to the five brain vesicles. (B) Left lateral view on the same embryonic brain and cranial nerves as depicted in A. CS, Carnegie stage; Die, diencephalon; Mes, mesencephalon; Met, metencephalon; Mye, myelencephalon; Tel, telencephalon.

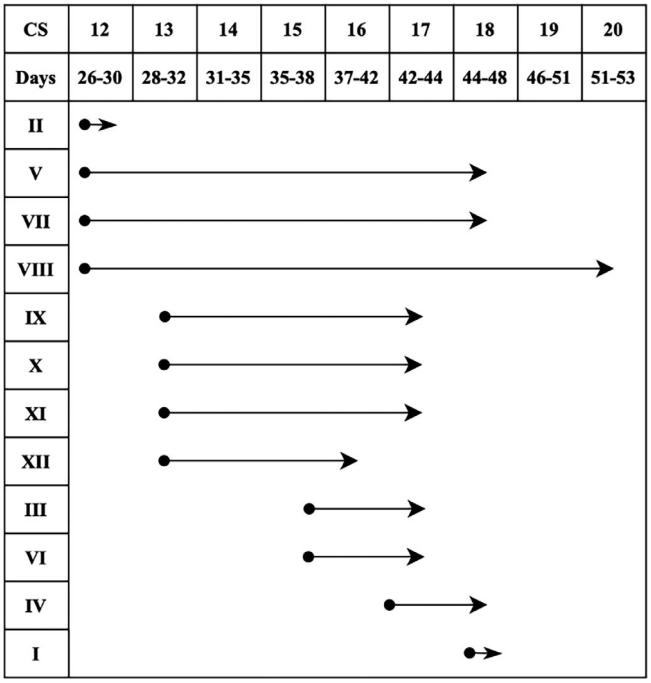

FIGURE 3 Cranial nerves: From first appearance to target organ. Visual representation of the first appearance of the cranial nerves per Carnegie stage shown as a dot and the stage in which they first reach their target organ or structure(s) as arrow-head. CN II develops as outgrowth of the neural tube and is therefore immediately connected with its target organ: the optic vesicle. Note the relatively early development of the pharyngeal arch nerves (CN V, VII, IX, and X). Also, most cranial nerves reach their target organ at or around CS 17 (42–44 days of development). CS, Carnegie stage.

initial development of CN V can be distinguished at CS 12 (Figure 1). At this stage, the trigeminal nerve appears as a primordium in connection with the metencephalon. At CS 14, the three main branches are already recognizable, respectively the ophthalmic nerve, the maxillary nerve, and the mandibular nerve (Table 1). In the following stages, the ophthalmic nerve develops ventrally toward the orbit, the maxillary nerve to the area between the nasal cavity and the maxilla and the mandibular nerve develops caudally coursing into the mandible.

In comparison to the rest of the developing brain, the relative growth of the cranial nerves increases in time in early CSs. Comparison of different embryos in different CSs did not reach statistical significance via ANOVA ($p = 0.13$). However, because of scarcity of this historical material, only a small number of embryos was included in this analysis ($n = 2$ per CS). One could identify a trend in which relative growth increases at early stages, peaks at CS 17 and slowly decreases afterwards (Figure 5).

4 | DISCUSSION

Most cranial nerves have completed their development early in embryology (i.e., CS 13–17). Agenesis and dysgenesis of cranial nerves is most crucial in CS 15–17. Therefore, the etiology of congenital cranial dysinnervation disorders (CCDDs) (e.g., Duane's retraction syndrome and Möbius syndrome) should be studied within these CSs. Interestingly, apart from the optic nerve which is merely an outgrowth of the diencephalon, the four pharyngeal arch nerves were the first cranial nerves to emerge in CS 12 and 13 (CN V, VII, IX, and X). The first cranial nerve (CN I) was, in contrary to what its name suggests, the last nerve to develop, in CS 18. Cranial nerve volumes increase early in embryology and one could identify a peak at CS 17, however only two specimens per CS were examined.

In this study, several discrete discrepancies with the literature were found. It should be noted that some variation between the existing literature and our findings is to be expected because there is age variation within CSs. No discrepancies with literature were found for CN II/IV/VI/VII/VIII/X and CN XII (O'Rahilly, 1975). Discrepancies were found for the remaining cranial nerves: CN I/III/V/IX and CN XI (O'Rahilly, 1975).

According to different studies, CN I initially appears at CS 16 (Müller & O'Rahilly, 1989; Müller & O'Rahilly, 2004). At CS 17, a

TABLE 1 Cranial nerves with corresponding Carnegie stages and target organ

CN	Appearance	Appearance of branch	Target organ nerve/branch	Reaching target organ
I	CS 18	None	Arises from the nasal epithelium	CS 18
II	Debatable	None	Arises from the retina	Instantly
III	CS 15/16	CS 17: inferior and superior branch of oculomotor nerve	Superior/inferior/medial rectus muscle	CS 17
IV	CS 16/17	None	Inferior oblique muscle	CS 18
V	CS 12	CS 14: maxillary nerve CS 14: mandibular nerve CS 14: ophthalmic nerve CS 17: inferior alveolar nerve CS 17: buccal nerve CS 17: lingual nerve CS 17: nasociliary nerve CS 17: frontal nerve CS 18: auriculotemporal nerve CS 19: infraorbital nerve CS 20: supratrochlear nerve CS 20: supraorbital nerve CS 21: lacrimal nerve	Oral epithelium and skin of the upper jaw (becomes upper lip) Tongue Divides into nasociliary, lacrimal, and frontal nerve Tympanic sulcus Lateral side of oral cavity Tongue Skin ventral to nasal epithelium Divides into supraorbital nerve and supratrochlear nerve Muscles of mastication Oral epithelium and skin of the lower jaw (becomes lower lip and chin) Pericranium Skin surrounding the eye Lacrimal gland (dorsal side of the eye)	CS 18 CS 19 CS 19 CS 18 CS 19 CS 19 CS 22 CS 22 CS 19 CS 19 CS 19 CS 20 CS 20
VI	CS 15/16	None	Lateral rectus muscle	CS 17
VII	CS 12/13	CS 15: somatomotor branch CS 15: chorda tympani CS 17: greater petrosal nerve	Muscles of facial expression Joins the lingual nerve of trigeminal nerve Pterygopalatine ganglion	CS 18 CS 18 CS 19
VIII	CS 12/13	CS 20: anterior ampullary nerve CS 20: lateral ampullary nerve CS 20: posterior ampullary nerve	Organ of Corti Connected with neural tube Ampullae	during fetal development CS 17 CS 20
IX	CS 13	None CS 17: tympanic branch[a]	Caudal part of the tongue	CS 17
X	CS 13	CS 16: superior laryngeal nerve CS 16: recurrent laryngeal nerve CS 17: esophageal plexus CS 17: pulmonary plexus CS 18: dorsal gastric branches CS 19: ventral gastric branches	Larynx Larynx Esophagus Lungs Dorsal side of stomach Ventral side of stomach	CS 17 CS 18 CS 17 CS 18 CS 18 CS 19
XI	CS 13/14	None	Sternocleidomastoid-trapezius muscles	CS 17
XII	CS 13	None	Muscles of the tongue (both intrinsic and extrinsic)	CS 16

Note: Table showing the Carnegie stage (CS) of the first appearance of the cranial nerves and of any potential branches. The target organs of these branches are shown, together with the CS when the target organs are reached.
[a]From literature.

bundle of fibers to the primordial olfactory bulbs is distinguishable (Bossy, 1980). In the current study, CN I was not perceived until CS 18. One stage earlier, some condensations can be recognized, but it cannot be determined with certainty if these represent initial CN I development. The first fibers of CN III have been reported to appear at CS 14 (Müller & O'Rahilly, 1988; O'Rahilly et al., 1984). This is not in accordance with the findings in the current study, where they appeared at CS 15/16.

According to Nanci & Ten Cate, the inferior alveolar and lingual branches of the mandibular nerve show initial development in a 6-week embryo (Nanci & Ten Cate, 2008), which is comparable to CS 16 or CS 17 (O'Rahilly, 1979). Pearson, however, describes first occurrence already at week five, which is comparable to CS 14 or CS 15 (Pearson, 1977). In our study, the two branches of the mandibular nerve were found in the CS 17 embryo.

The findings in the literature regarding CN IX development are comparable to the findings in this study, except for the tympanic branch. It is mentioned that this nerve appears when embryos reach 12–14 millimeters in length (CS 17/18) (Streeter, 1908). However, this nerve was not yet distinguishable in our study.

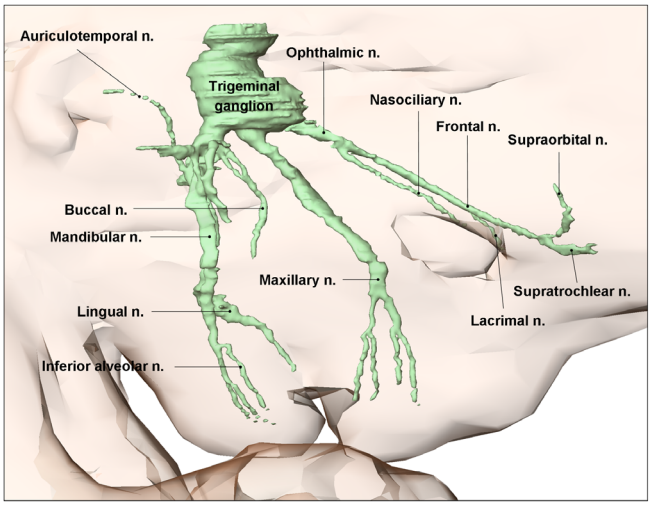

FIGURE 4 The intricate branching of the right trigeminal nerve in a human embryo. A 3D reconstruction of the CN V is provided in CS 23 specimen #950 (56–60 days of development). Right lateral view. Note how impressively intricate its branches are already developed in this embryo with a Crown-Rump-Length of only 23.79 mm. CS, Carnegie stage.

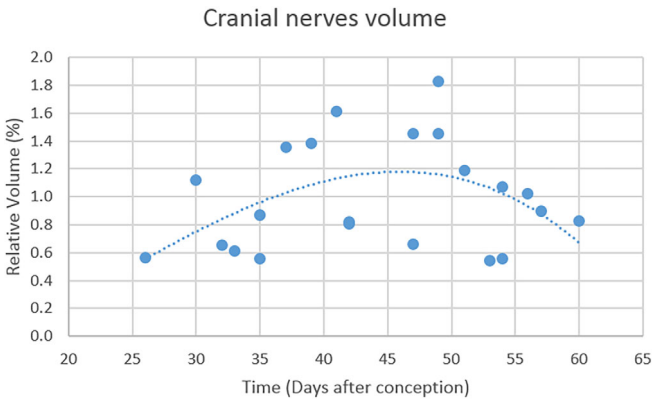

FIGURE 5 Relative volume of the cranial nerves. Visual representation of the relative volume of the cranial nerves compared to the volume of the rest of the brain. Although a peak at CS 17 (42–44 days of development) could be assumed, no statistical significance was reached ($p = 0.13$). CS, Carnegie stage.

All findings in this study regarding CN XI development are comparable with other publications, except for the timing when CN XI reaches the mesenchymal condensation that gives rise to the sternocleidomastoid and trapezius muscles. According to literature, this occurs at the end of the fourth week (Johal et al., 2019), comparable to CS 14 or 15 (O'Rahilly, 1979). However, in our study this event took place no earlier than at CS 17. This discrepancy might be attributed to the use of different human tissue (Carnegie collection versus other human tissue).

In our study, we found that the relative growth of cranial nerves compared to the total volume of the embryo increases initially. A peak is suggested at CS 17, however no statistical significance was reached ($p = 0.13$). This peak could imply that cranial nerves increase relatively quick in volume until they reach their target organs, after which their relative volume decreases again toward their small size as known from adult anatomy. We assume that cranial nerves are typically vulnerable to teratogens (e.g., medication or radiation) up until the stage in which they reach their target organ. More research is needed to compare our data to specimens from other collections. Currently, no previous studies on this topic are yet conducted to compare our results with.

In all, the temporal and spatial embryonic development of the human cranial nerves was identified in this study. With this 3D framework, future studies can relate the development of the human cranial nerves to possible anatomical variants, abnormal development, or developmental timing of these structures.

5 | CONCLUSIONS

This is the first time that detailed volume measurements of cranial nerves in the human embryonic period have been presented. In general, most development of the cranial nerves is completed early in embryology (CS 13–17). Additional research is needed that focuses on specific neurological pathologies.

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REFERENCES

Bossy, J. (1980). Development of olfactory and related structures in staged human embryos. *Anatomy and Embryology*, *161*(2), 225–236.

Chieco, P., Jonker, A., de Boer, B. A., Ruijter, J. M., & Van Noorden, C. J. (2013). Image cytometry: Protocols for 2D and 3D quantification in microscopic images. *Progress in Histochemistry and Cytochemistry*, *47*(4), 211–333.

De Bakker, B. S., de Jong, K. H., Hagoort, J., De Bree, K., Besselink, C. T., de Kanter, F. E. C., Veldhuis, T., Bais, B., Schildmeijer, R., Ruijter, J. M., Oostra, R. J., & Moorman, A. F. (2016). An interactive three-dimensional digital atlas and quantitative database of human development. *Science*, *354*(6315).

De Bakker, B. S., de Jong, K. H., Hagoort, J., Oostra, R. J., & Moorman, A. F. (2012). Towards a 3-dimensional atlas of the developing human embryo: The Amsterdam experience. *Reproductive Toxicology*, *34*(2), 225–236.

His, W. (1895). *Die anatomische nomenclatur*. Vogel.

Iwanaga, J., Singh, V., Takeda, S., Ogeng'o, J., Kim, H.-J., Moryś, J., Ravi, K. S., Ribatti, D., Trainor, P. A., Sañudo, J. R., Apaydin, N., Sharma, A., Smith, H. F., Walocha, J. A., Hegazy, A. M. S., Duparc, F., Paulsen, F., Del Sol, M., Adds, P., … Tubbs, R. S. (2022). Standardized statement for the ethical use of human cadaveric tissues in anatomy research papers: Recommendations from anatomical journal editors-in-chief. *Clinical Anatomy*, *35*, 526–528.

Johal, J., Iwanaga, J., Tubbs, K., Loukas, M., Oskouian, R. J., & Tubbs, R. S. (2019). The accessory nerve: A comprehensive review of its anatomy, development, variations, landmarks and clinical considerations. *The Anatomical Record*, *302*(4), 620–629.

Keibel, F., & Elze, C. (1908). *Normal plates of the development of the human embryo (homo sapiens)*. Fisher.

Keibel, F., & Mall, F. P. (1910). *Manuel of human embryology*. Lippincott Company.

Müller, F., & O'Rahilly, R. (1988). The first appearance of the future cerebral hemispheres in the human embryo at stage 14. *Anatomy Embryology*, *177*(6), 495–511.

Müller, F., & O'Rahilly, R. (1989). The human brain at stage 16, including the initial evagination of the neurohypophysis. *Anatomy Embryology*, *179*(6), 551–569.

Müller, F., & O'Rahilly, R. (2004). Olfactory structures in staged human embryos. *Cells, Tissues, Organs*, *178*(2), 93–116.

Nanci, A., & Ten Cate, A. R. (2008). *Ten Cate's oral histology: Development, structure, and function*. Elsevier Health Sciences.

O'Rahilly, R. (1975). *A color atlas of human embryology*. W.B. Saunders.

O'Rahilly, R. (1979). Early human development and the chief sources of information on staged human embryos. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, *9*(4), 273–280.

O'Rahilly, R., Müller, F., Hutchins, G. M., & Moore, G. W. (1984). Computer ranking of the sequence of appearance of 100 features of the brain and related structures in staged human embryos during the first 5 weeks of development. *The American Journal of Anatomy*, *171*(3), 243–257.

Pearson, A. A. (1977). The early innervation of the developing deciduous teeth. *Journal of Anatomy*, *123*, 563–577.

Streeter, G. L. (1908). The peripheral nervous system in the human embryo at the end of the first month (10 mm). *The American Journal of Anatomy*, *8*, 285–301.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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