

## Early fetal development of the human cerebellum

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**Abstract** Early cerebellum development in humans is poorly understood. The present study histologically examined sections from 20 human embryos and fetuses at 6 weeks (12–16 mm crown-rump length (CRL); 4 specimens), 7–9 weeks (21–39 mm CRL; 8 specimens), 11–12 weeks (70–90 mm CRL; 4 specimens) and 15–16 weeks (110–130 mm CRL; 4 specimens). During 7–9 weeks (approximate CRL 28 mm), the rhombic lip (a pair of thickenings of the alar plate) protruded dorsally,

bent laterally, extended ventrolaterally and fused with the medially located midbrain. During that process, the primitive choroid plexus appeared to become involved in the cerebellar hemisphere to form a centrally located eosinophilic matrix. At that stage, the inferior olive had already developed in the thick medulla. Thus, the term ‘bulbopontine extension’ may represent an erroneous labeling of a caudal part of the rhombic lip. The cerebellar vermis developed much later than the hemisphere possibly from a midline dark cell cluster near the aqueduct. In the midline area after 12 weeks (80 mm CRL), the growing bilateral hemispheres seem to provide mechanical stress such as rotation and shear that cause the development of several fissures much deeper than those on the hemisphere. The rapidly growing surface germinal layer may be a minor contributor to this vermian fissure formation. The vermian fissures seem to enable inside involvement of the surface germinal cells, and to induce cytodifferentiation of the vermis. Consequently, in the early stages, it appears that the cerebellar hemisphere and vermis develop independently of each other.

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### Introduction

Studies on embryological development of the human cerebellum have generally used sagittal sections [6, 7]. The use of such sections has provided detailed information regarding fissure formation and the topographical relationship between the primitive cerebellum and the fourth ventricle. Sagittal views have also been the basis of schematic diagrams showing the stages of cerebellar histogenesis [18].

The cerebellar hemisphere develops from a pair of thickenings at the lateral ends of the mesencephalic alar plate, the so-called rhombic lip [5]. Although Utsunomiya et al. [16] postulated a model in which the growing rhombic lip fuses with the alar plate to form the cerebellar hemisphere, histological investigations may not be able to show such a process because it is difficult to observe the rhombic lip in sagittal sections. Yamamoto et al. [19] and Currle et al. [3] postulated that the choroid plexus accelerated or induced development of the cerebellar hemisphere. However, there are no human study data to support that contention.

The cerebellar vermis occupies the middle region that connects both cerebellar hemispheres. However, embryological understanding of the human vermis is mostly restricted to that relating to the Dandy–Walker malformation and its variations because those pathologies are characterized by a lack of vermis [1, 6, 13, 15]. Vermian development was also described in a much earlier limited classical study [7]. Chick and mouse studies suggest that the vermis develops from a pair of separate anlagen [4, 9, 10]. The origin of the human vermis remains unknown. Utsunomiya et al. [16] considered it unlikely that a midline fusion of the vermian anlage occurs in humans.

The present study investigated human fetal cerebellum development using horizontal and frontal sections, with a particular emphasis on the midline vermis. The embryos used were at much earlier stages than those used in recent excellent studies of human cerebellar histogenesis [8, 12, 17].

## Materials and methods

The present study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). The study involved histological examination of paraffin-embedded tissue taken from a total of 20 embryos or fetuses. The embryos/fetuses were at four stages of development: 6 weeks (12–16 mm crown-rump length (CRL); 4 specimens), 7–9 weeks (21–39 mm CRL; 8 specimens), 11–12 weeks (70–90 mm CRL; 4 specimens) and 15–16 weeks (110–130 mm CRL; 4 specimens) of ovulatory age. All specimens were part of a large collection at the Embryology Institute of the Universidad Complutense, Madrid, and were the products of miscarriages and ectopic pregnancies that were managed at the Department of Obstetrics of the University. Approval for the study was granted by the university ethics committee. Given the specimens were the result of miscarriages and ectopic pregnancies, it is possible that they had associated pathologies. To minimize any effect of such a potential confounder, the morphologies described are those commonly observed at each developmental stage.

The specimens were processed using routine paraffin-embedded histology procedures. Sixteen specimens were cut horizontally, and four were cut frontally (the group at 15–16 weeks) because they were too large to be cut horizontally. Sections were 5- $\mu$ m thick, and made at intervals of 20–100  $\mu$ m depending on specimen size. The sectional planes (horizontal or frontal) used were those described in our recent study on the primitive meninges [2]. Depending on specimen size, observations were required on approximately 50–200 sections, including almost the entire mesencephalic flexure region surrounded by the primitive thalamus, midbrain, pons and medulla. All sections were stained with hematoxylin and eosin (HE).

## Results

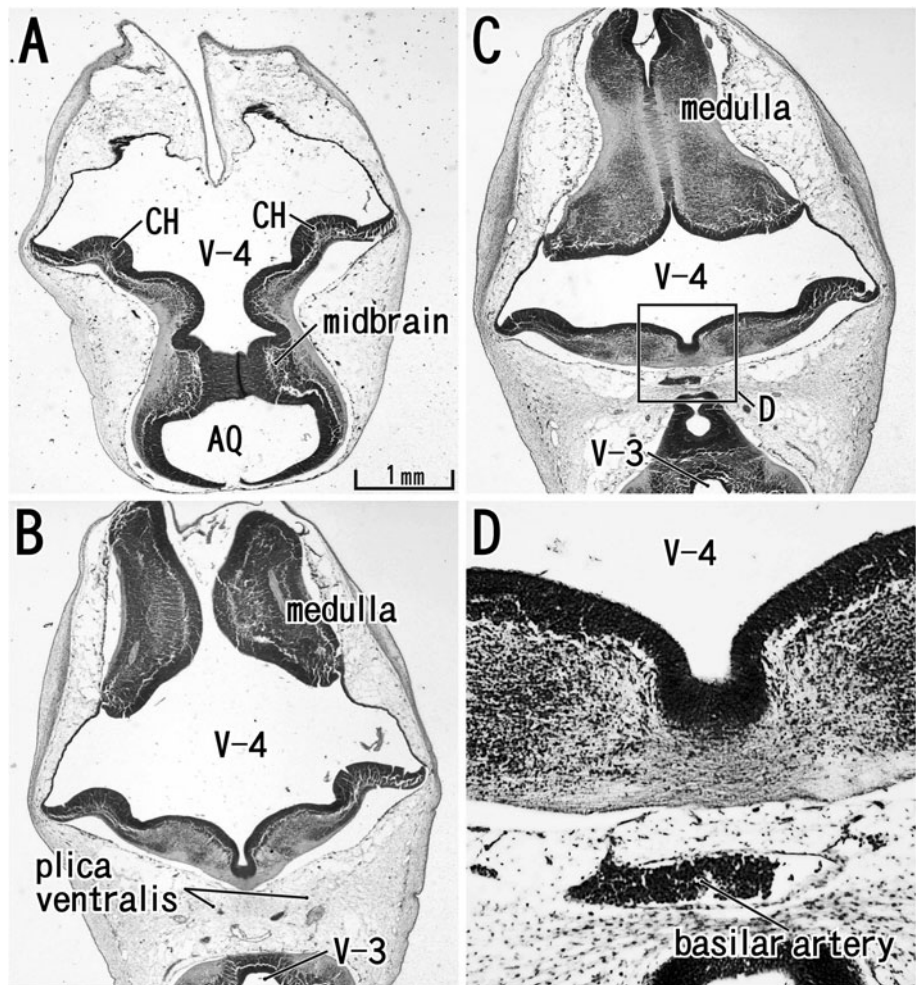
### At 6 weeks (CRL 12–16 mm)

The anlage of the cerebellum was first identified as a pair of thickenings on the lateral site of the alar plate that faced the fourth ventricle (Fig. 1a, b). However, the initial rhombic lip was not located at the lateral end of the alar plate. The proliferating neuroepithelial layer or ependymal cell lining of the ventricles had already fused at a level of the midbrain or mesencephalon. A specific cell cluster appeared later at that site (see “The 11–12 weeks” description). The medulla was already identifiable as a thick tube-like structure in the caudal side of the fourth ventricle, opening widely to the dorsal side of the brain (Fig. 1c). At the level of the initial rhombic lip, the midline area corresponded to the floor plate comprising transversely oriented cells (Fig. 1d); it did not correspond to the dorsal midline but to the ventral midline.

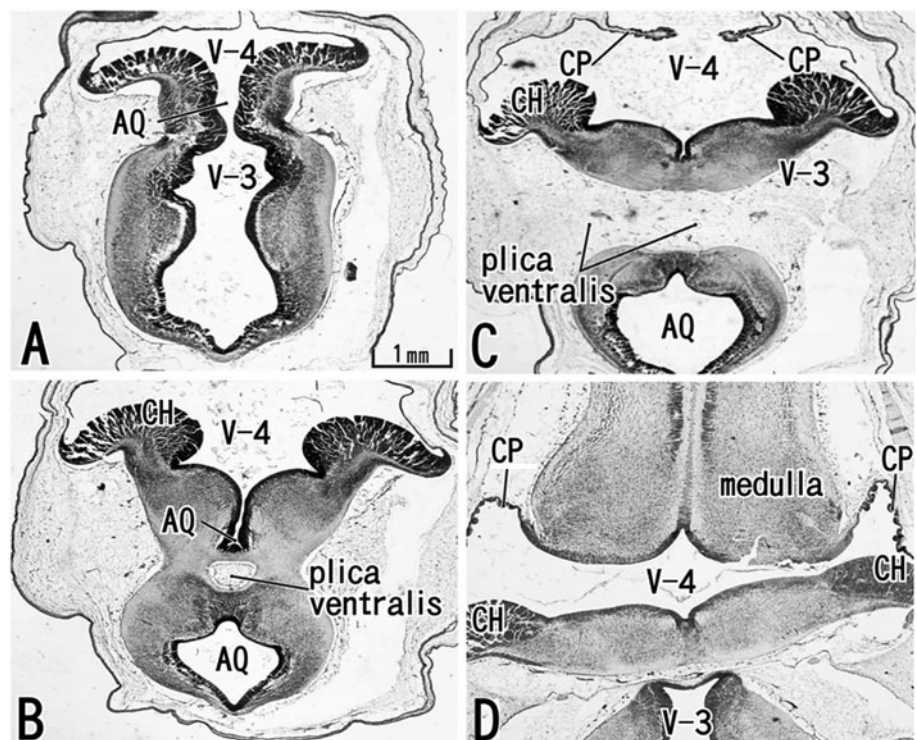
### At 7–9 weeks (CRL 21–39 mm)

During this stage, the rhombic lip displayed dynamic development into the primitive cerebellar hemisphere. The pair of alar plate thickenings grew to form a pair of rhombic lips (Figs. 2, 3, 4, 5). The rhombic lip protruded dorsally, bent laterally and extended ventrolaterally around the midbrain (Figs. 3, 4). This process appeared to occur from the caudal to cranial sites according to a difference between cranio-caudal levels in the brain. An eosinophilic matrix was evident between the alar plate and the ventrolaterally extending rhombic lip (Figs. 3, 4). Notably, the extending rhombic lip connected with the primitive choroid plexus and involved it into the centrally located eosinophilic matrix (Fig. 3d–f). In one fetus (CRL 27 mm), the bilateral lips aligned together in the dorsal midline, but they were not fused.

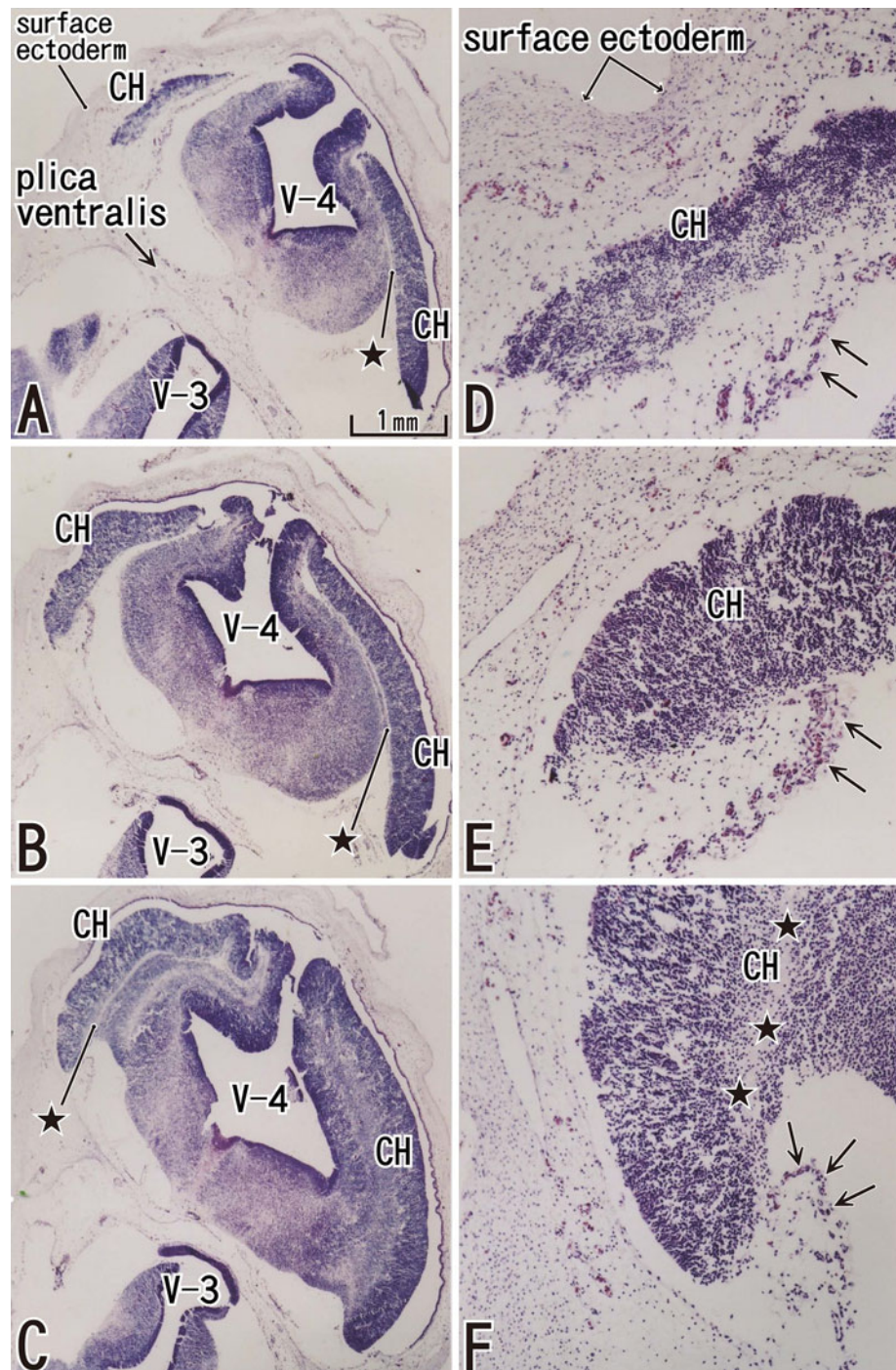
**Fig. 1** Horizontal sections of the midbrain and medulla in a fetus with a 14 mm CRL. Panel **a** is the most cranial, and panel **c** the most caudal. Panel **d** shows a higher magnification view of the midline structure (*floor plate*) corresponding to the *square* shown in panel **c**. Anlage of the cerebellar hemisphere (CH) is identified bilaterally as thickening of the alar plate facing the fourth ventricle (V-4). Note the medulla is already a large mass. AQ future aqueduct in the midbrain, V-3 the third ventricle. The *upper side* of the figure corresponds to the dorsal side of the brain



**Fig. 2** Horizontal sections of the midbrain and medulla in a fetus with a 21 mm CRL. Panel **a** is the most cranial, and panel **d** the most caudal. Panel **b** corresponds to the top of the plica ventralis protruding into the mesencephalic flexure (the future interpeduncular fossa). In panels **b** and **c**, anlage of the cerebellar hemisphere (CH) or the so-called “rhombic lip” is identified bilaterally as thickening of the alar plate facing the fourth ventricle (V-4). Note the developed medulla (panel **d**). AQ future aqueduct in the midbrain, CP choroid plexus; V-3 the third ventricle. The *upper side* of the figure corresponds to the dorsal side of the brain



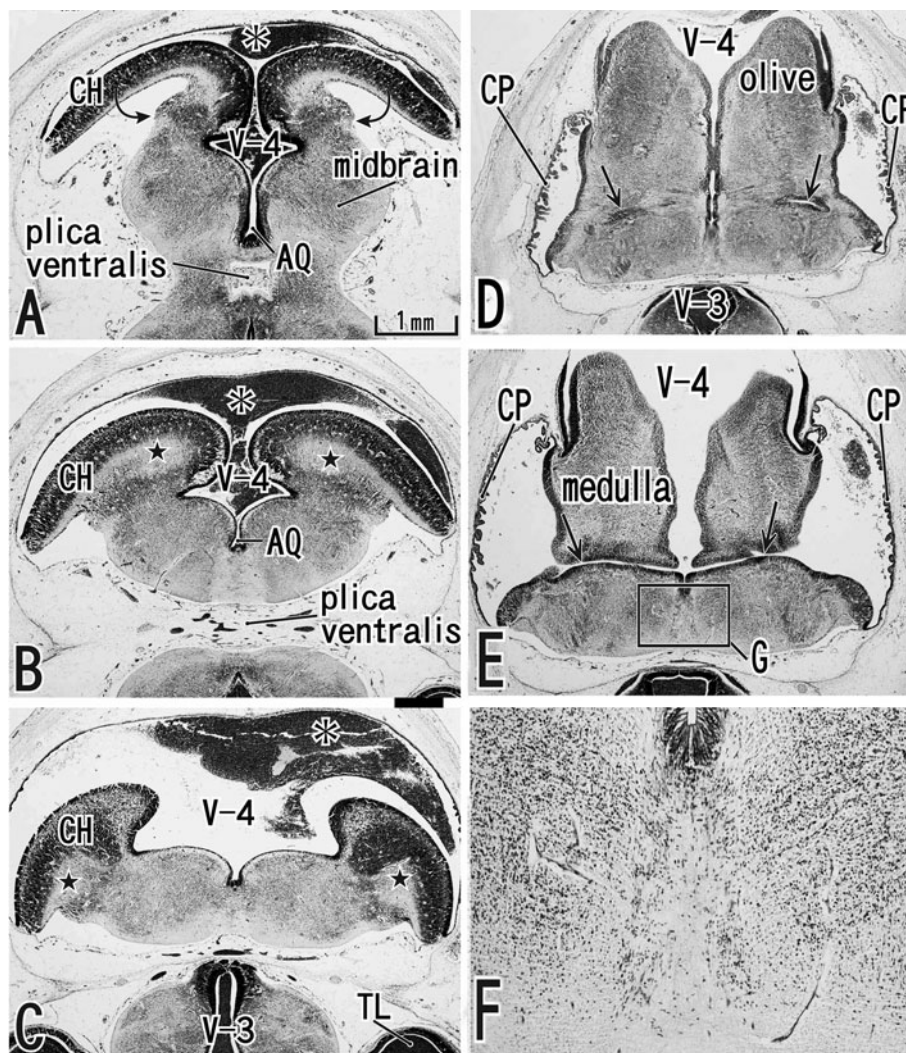
**Fig. 3** Horizontal sections showing lateral bending of the cerebellar extensions and fusion with the midbrain in a fetus with a 28 mm CRL. Panel **a** is the most cranial, and panel **c** the most caudal. Due to bending near the dorsal midline, the bilateral extensions for the cerebellar hemisphere (CH) extend ventrolaterally. Panels **d–f** are higher magnification views of the hemisphere (*left-hand side*) shown in panels **a–c**, respectively. Primitive choroid plexus tissues connect with the developing cerebellum (*arrows* in panels **d–f**). The choroidal tissues appear to continue to an eosinophilic matrix in the cerebellar hemisphere (*stars* in panels **a, b, c** and **f**). The other abbreviations are as per Fig. 2 and the *upper side* of the figure corresponds to the dorsal side of the brain



The basal plate thickened, and the transversely oriented cells were still observed in the floor plate along the ventral midline. However, those cells tended to disappear in the late period of this stage (Fig. 4f). On the caudal side of the rhombic lip, the medulla became thick (Fig. 2d) and protruded dorsally (Fig. 4d). The choroid plexus connected between the medulla and developing cerebellar hemisphere (Figs. 4d, e and 5c, d). Within the medulla, the inferior

olive was identified as a pair of cell clusters (Figs. 4d, 5d). However, the olive and developing cerebellar hemisphere were almost always separated by a space or fissure continuous with the fourth ventricle (Figs. 4, 5). In one fetus (CRL 35 mm), the dorsally protruded medulla appeared to attach to the growing cerebellar hemisphere, but the choroid plexus interposed between them. Therefore, the rhombic lip itself never connected with the olive.

**Fig. 4** Horizontal sections of the midbrain and medulla in a fetus with a 29 mm CRL. Panel **a** is the most cranial, and panel **e** the most caudal. Panel **a** corresponds to the top of the plica ventralis protruding into the mesencephalic flexure. The bilateral extensions for the cerebellar hemisphere (CH) are going through a process of attaching to the midbrain (curved arrows in panel **a**). After attachment, the hemisphere contains an eosinophilic matrix (stars in panels **b** and **c**). The primitive inferior olive is identified in the medulla (panel **e**). Arrows in panels **d** and **e** indicate a fissure between the medulla and cerebellum. Panel **g** shows a higher magnification view of the square in panel **f** highlighting the future vermis. The developing choroid plexus (CP) is seen at the lateral sites in panels **e** and **f**. Asterisks indicate bleeding. AQ aqueduct of the midbrain, TL cerebral temporal lobe. The upper side of the figure corresponds to the dorsal side of the brain



At 11–12 weeks (CRL 70–90 mm)

The cerebellar hemisphere became as thick as the midbrain. In the hemisphere, a laminar configuration became evident but the central eosinophilic matrix remained present. Fissures of the future vermis appeared in the midline area (Fig. 6): the developing fissures provided island-like structures in horizontal sections. The hemisphere and vermis, including the surfaces of the fissures, were covered by the external germinal cell layer. A cluster of hematoxylin-positive, large dark cells was evident in the dorsal midline area facing the fourth ventricle (Fig. 6b, c). Cranially, this group of cells continued to the ependymal cell lining of the ventricles at the opening of the aqueduct into the fourth ventricle.

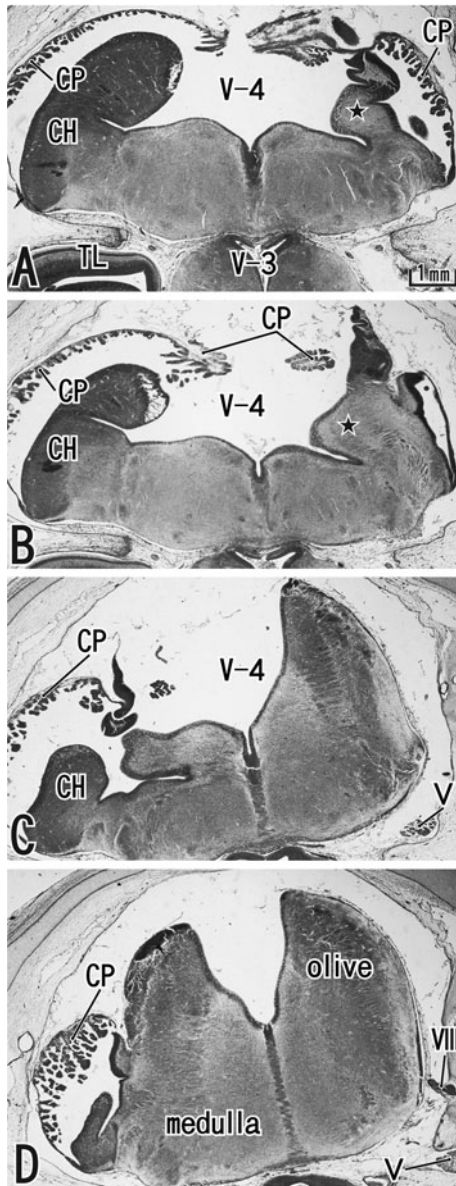
At 15–16 weeks (CRL 110–130 mm)

The cerebellar hemisphere contained the primitive dentate nucleus. The nodule and flocculus were identified. The

vermis became as thick as the hemisphere and it accompanied several deep fissures (Fig. 7). The developing fissures provided island-like structures in frontal sections. Although each fissure was covered by the external germinal layer, the cells did not invade the vermicular substrate or cortex. Along the inner side of the external germinal layer another cell layer became evident (possibly the external granular layer; Fig. 7e). However, the architecture of the vermis remained much simpler than that of the hemisphere. The cluster of hematoxylin-positive, dark cells was also seen along the dorsal midline facing a border area between the aqueduct and fourth ventricle (Fig. 7). The cells were similar to the superficial external germinal cells lining the cerebellum.

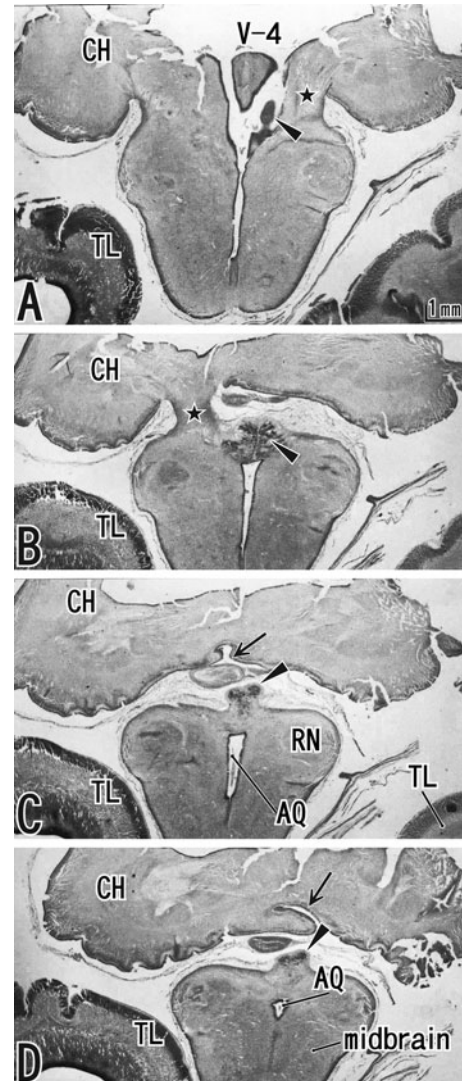
## Discussion

The present study used staged transverse histology to examine early human fetal cerebellum development.



**Fig. 5** Horizontal sections of the midbrain and medulla in a fetus with a 39 mm CRL. Panel **a** is the most cranial, and panel **d** the most caudal. On the caudal side of the developing cerebellar hemisphere (CH), the primitive inferior cerebellar peduncle (*star* in panels **A** and **B**) continues to the medulla. The primitive inferior olive is clearly identified in the medulla (panel **d**). The trigeminal and vestibulocochlear nerve roots (V, VIII) are seen in panels **c** and **d**. Other abbreviations are as per Fig. 4 and the *upper side* of the figure corresponds to the dorsal side of the brain

We observed rhombic lip fusion with the alar plate to form the cerebellar hemisphere following bending of the lips at 7–9 weeks. This finding supports the model postulated by Utsunomiya et al. [16]. We found that the fusion plane seemed to correspond to a centrally located eosinophilic matrix observed after fusion. Notably, fusion between the lip and plate was likely to involve the primitive choroid plexus inside of the cerebellar hemisphere, possibly into

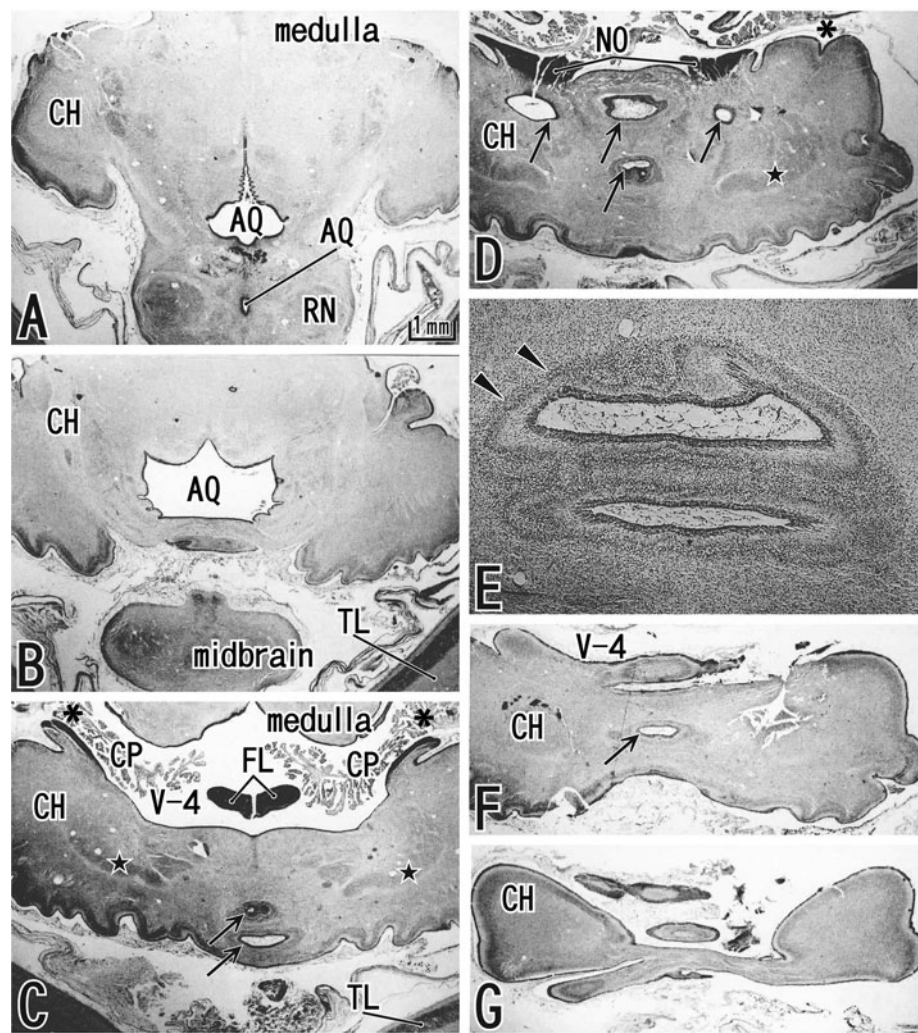


**Fig. 6** Almost frontal sections of the midbrain and medulla in a fetus with an 80 mm CRL. Panel **a** is the most posterior, while panel **d** the most anterior. Panels **a** and **b** include the superior cerebellar peduncle (*stars*). The cerebellar hemisphere displays a laminar architecture. The developing vermis contains a fissure (*arrows* in panels **c** and **d**). Note a cluster of *dark cells* in the *midline* (*arrowhead*). RN red nucleus. Other abbreviations are as per Fig. 4 and the *upper side* of the figure corresponds to the dorsal side of the brain

the eosinophilic matrix. Yamamoto et al. [19] and Currie et al. [3] demonstrated experimentally that the choroid plexus induced and accelerated development of the cerebellar hemisphere through secretion of retinoic acid. The present findings suggest a more direct contribution of the choroid plexus to cerebellar development.

Chick and mouse studies have suggested that the vermis develops from a pair of separate anlagen [4, 9, 10]. We did not observe such a dorsal midline fusion of the cerebellum, although a type of dorsal fusion should occur when the aqueduct forms in the early stage. Instead, in the later stage, a cluster of hematoxylin-positive, large dark cells,

**Fig. 7** Almost frontal sections of the midbrain and medulla in a fetus with a 125 mm CRL. Panel **a** is the most anterior, while panel **g** the most posterior. In the vermis, developing fissures provide island-like structures (arrows in panels **c**, **d** and **f**). Panel **e** is a higher magnification view of the islands at the intermediate level between panels **d** and **f**. Panel **e** shows the island surrounded by the ependymal cells and a newly developed granular layer (arrowheads). The flocculus (FL) and nodulus (NO) appear as cerebellum protrusions in panels **C** and **D**, respectively. Laminar structures are clearly identified in the cerebellar hemisphere (CH). Stars indicate the future dentate nucleus. Asterisks in panels **c** and **d** indicate the future lateral recess. AQ Aqueduct of the midbrain, CP choroid plexus, RN red nucleus, TL cerebral temporal lobe, V-4 fourth ventricle. The upper side of the figure corresponds to the dorsal side of the brain



continuous with the ependymal cell layer (proliferating neuroepithelium), became evident in the dorsal midline area at the opening of the aqueduct to the fourth ventricle. The vermis differentiation occurred much later than rhombic lip development into the cerebellar hemisphere. Thus, in the early stage, the cerebellar hemisphere and vermis seem to develop independently of each other. Because the vermis deep fissure formation occurred on the immediately caudal side of the aforementioned cell cluster, it appeared to correspond to the anterior margin of the vermician anlage to initiate the development. Moreover, the cluster of dark cells, mostly or partly, is likely to differentiate into the vermis when the caudal end of the aqueduct is limited by the cell cluster. The suggested vermician development independent of the hemisphere seems to explain a fact that Dandy–Walker malformation usually accompanies the normal hemisphere [6, 15].

Early vermis development was characterized by the formation of several deep fissures. The mechanisms underlying the formation of those fissures are yet to be

identified. The fissures appear to be too deep to be a product of proliferation and growth of the surface germinal layer cells, and the morphology of the germinal cell lining was similar for sites in and outside of the fissure. Histology findings provided no evidence of germinal cell invasion along the fissures. Therefore, we propose that the development of the deep fissures was the result of another mechanism, such as mechanical stress. Kapur et al. [6] suggested that the cerebellar hemisphere rotated due to hydrostatic forces in the dilated fourth ventricle in a pathological condition. Likewise, Zalei et al. [20] hypothesized rotation of the vermis in the enlarged cisterna magna. Therefore, even in normal development, the rapidly growing bilateral hemispheres are likely to provide rotation and shear stress to form vermician fissures much deeper than those on the hemisphere in the early stage. Although the major force behind fissure development may be mechanical, we cannot rule out a contribution from the germinal layer. Similarly, the developing cerebellar nodule and flocculus

may contribute. However, the latter appears less likely since those two structures only became evident 3–4 weeks after fissure formation.

Textbooks [5, 14] indicate that the medullary olive and the pontine nuclei develop from the “bulbo-pontine extension”. Although those structures carry close fiber connections with the cerebellum, previous reports on cerebellar development did not describe this specific extension of the fetal brain stem. Millen et al. [11] considered that the lateral pontine and olivary nuclei originate from the rhombic lip. However, we did not find any connection between the lip and olive. The present findings suggest that the term bulbo-pontine extension may represent an erroneous labeling of a caudal part of the rhombic lip. Likewise, at the rostral part, a concept of the rhombic lip fusion, such as a zip fastener, seems to be a product of imagination because the large cerebellar hemisphere extended far laterally without any folding (see also the vermian development in the second paragraph of the “Discussion”).

The present study had some limitations. Identification of the initial vermian was difficult since sagittal sections were not used. Thus, the findings may suggest that the vermian “suddenly” appears between 9 and 11 weeks of gestation. Because our observations started from the stage with the rhombic lip, we were not able to make a comment on a hypothesis that the primordium of the cerebellar hemisphere is initially unpaired [16]. The present study did not include immunohistochemistry, which limited the description of intracerebellar laminar architecture although many legends are lost or changed during the postmortem procedure. Thus, we should avoid over discussion especially on the clinical relevance.

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