The growth and developmental of the myodural bridge and its associated structures in the human fetus

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Abstract

The myodural bridge (MDB) is a dense connective tissue structure between the suboccipital musculature and the spinal dura mater (SDM). However, few reports on the development and maturation of the human MDB. 30 head and neck specimens from human fetuses (F) from 12–40 weeks (W) were made into histological sections. The F12W sections evidenced that the SDM dominated by fibroblasts, attached to the posterior atlantoaxial membrane (PAAM) which completely sealed the atlantoaxial space. In the F13W stage, myofibrils of the suboccipital muscle fibers increased significantly in number. At the F14W stage, a gap was observed at the caudal end of the PAAM. Numerous myodural bridge-like structures were observed blending into the SDM through the gap. At the F15W stage, the MDB fibers originated between the posterior arch of atlas (C1) and the obliquus capitis inferior muscle, and extended forward and downward into the spinal canal through the atlantoaxial gap. Starting at the F21W stage, the MDB fibers were observed to pass through the atlantoaxial interspace and radially attach to the SDM. The present study adds to the knowledge base of developmental morphology research of the human embryonic MDB and provides a developmental morphological basis for its potential functionality.

Background

In 1995, Hack et al.introduced the term myodural bridge (MDB)[1]. The MDB is a dense fibrous structure passing through both the posterior atlanto-occipital interspace[2] as well as the posterior atlanto-axial interspace[3]. The MDB connects several of the suboccipital muscles and ligaments to the spinal dura mater (SDM). The relevant suboccipital muscles are the rectus capitis posterior minor (RCPmi), the rectus capitis posterior major (RCPma), and the obliquus capitis inferior (OCI)[4,5], and the ligaments include the ligamentum nuchae (LN)[6,7], the vertebral dura ligament (VDL) and the to be named ligament (TBNL)[8]. Recently, numerous papers have described the MDB in humans. Yuan et al. described four different attachment types of the rectus capitis posterior minor (RCPmi) muscle[9]. Zheng et al. found that the MDB of humans is primarily formed by type I collagen fibers, which are arranged in parallel[10]. Kitamura et al. described suboccipital fascial structures in human fetuses[11]. Zheng et al. proposed that the MDB originating from the suboccipital muscles formed a functional unit that they termed the ‘Myodural Bridge Complex’ (MDBC)[12]. Jiang et al. described the ultra-microstructure of the human MDB via scanning electron microscopy[13].

Various researchers have suggested that the MDB may has significant physiological functions. For example, the MDB was described to be associated with the transmission of proprioception[14,15], to keep the subarachnoid space and the posterior cerebellomedullary cistern patent during head movements[16]. Moreover, the MDB has been described as one of the factors which potentially may affect the dynamic circulation of cerebrospinal fluid (CSF)[8,17].

However, to date there have been only a few limited studies on the developmental morphology of the human MDB, Thus, the relationship between the development of the MDB and the suboccipital musculature remains unclear. In the present study, histological sections with hematoxylin eosin staining were used to describe the development of the MDB and its muscular associations in the human fetus. The results of the present study will provide a baseline of new knowledge to support future research on the function of the human MDB.

Results

Histological study of the fetus at 12 to 13 weeks of gestation (Fig. 1a, b)

Examining the sagittal sections obtained from the suboccipital region of 12-week gestation fetuses, the dura mater appeared to be thin and composed of multiple layers of thin and wavy fiber bundles. At this stage of development, there appeared to be primarily fibroblasts each containing a large nucleus as well as obvious nucleoli in the spinal dura mater (Fig.1ai). The connective tissue composing both the MDB and the posterior atlanto-axial membrane (PAAM) appeared to be relatively loose, and primarily composed of fibroblasts. We observed numerous short fiber bundles in the PAAM that appeared disordered with no obvious directionality (Fig.1aii). From 12 weeks onward, muscle tissue was readily observed. However, the muscle fibers appeared to be relatively less numerous and loose and were primarily in the form of myotubes or developing skeletal muscle fibers with a tubular appearance. Moreover, at this stage of development we observed that there were numerous nuclei and some myofibrils beginning to be arranged in parallel (Fig. 1a).

At 13 weeks, both the quantity of connective tissue in suboccipital region and the thickness of the dura mater increased. Additionally, at this stage of development, the fiber bundles appeared to be thicker, and the cells contained within them being mainly fibrocytes. These fiber bundles appeared to run in a wave-like form (Fig. 1b). Moreover, the nucleus appeared to be small and deeply stained, and the nucleoli were not obvious. In comparison to the 12 week specimens, the fibers of the MDB and PAAM at 13 weeks appeared to be denser. Although these cells were observed to be primarily fibroblasts, the number of wavy fiber bundles increased, and their arrangement became more orderly (Fig.1b). Within the suboccipital muscles, the myofibrils became more numerous and denser when compared to the 12 week specimens, and numerous nuclei were still observed (Fig. 1b).

Histological study of the fetus at 14 to 15 weeks of gestation (Fig. 1c, d)

From the 14th week onward, the spinal dura gradually separates from the posterior wall of the spinal canal, and an epidural space appears (Fig. 1c, d). The dura mater is now very dense, and includes many typical fibrocytes with small deeply stained nuclei. There also appeared inconspicuous nucleoli and varying thicknesses of the fiber bundles which were oriented in a wavy shape (Fig. 1c). At this stage, the MDB fibers are very thin and connected to the dura mater through the epidural space. The fibers of the MDB and PAAM have now lined up in parallel. Within the fibers, the fibroblasts still dominated (Fig. 1c). Considering the suboccipital muscles, no obvious change in the number of myofibrils was observed (Fig. 1c -d).

Histological study of the fetus at 19 weeks of gestation (Fig. 2a)

At 19 weeks, the thickness of the dura mater continued to increase (Fig. 2a). However, the fibers in the posterior atlanto-axial interspace now evidenced a unique shape. Although it is connected to the dura mater, the fibers of the PAAM do not extend from the muscle to the dura mater run longitudinally between C1 and C2. The cells in the MDB and PAAM are still dominated by fibroblasts (Fig. 2a). At this stage, the cells nuclei of the suboccipital muscles have now been deflected to one side, and the myotubes are filled with myofibrils. (Fig. 2a).

Histological study of the fetus from 21 to 30 weeks of gestation (Fig. 2b, c, d)

After 21 weeks, the morphological characteristics of the suboccipital region have stabilized. The fibers of the dura mater, MDB, and PAAM are have now all become denser. The cells within these structures have now become fibrocytes, and their fiber bundles are arranged in parallel. The suboccipital muscles have also become fuller (Fig. 2 b, c, d). Moreover, after observing the suboccipital muscles in different sections, it can be observed that the dense MDB fibers have the potential to pull the dura mater away from the spinal cord. In the section showing the To Be Named Ligament (TBNL), the posterior atlanto-axial interspace is now almost completely open with only a few fibers, and the dura mater is hardly stretched (Fig. 2c).

Histological study of the fetuses after 31 weeks of gestation (Fig. 3 a-d)

After 31 weeks, the development of the suboccipital region has stabilized and the morphology of the cells is mature. The cells of the dura mater and MDB have now differentiated into fibrocytes. As the fetus develops, the fibers become thicker and denser. The suboccipital muscles have matured, as well. Each muscle fiber has become fuller, and the intercellular space between muscle fibers has become smaller.

Discussion

Hack et al. (1995) described the MDB as a dense fibrous tissue that connects the rectus capitis posterior minor (RCPmi) muscle to the cervical spinal dura mater (SDM)[1]. Over the past 20 years, studies on the MDB have included anatomy, histology, and clinical pathology. However. few studies have been done on the development and maturation of the MDB in human fetuses. To fully understand the structure and function of the MDB, it is necessary to explore the growth and development of the MDB and its related structures (the spinal dura mater and the suboccipital musculature).

The dura mater differentiates progressively from 12 weeks of gestation to 40 weeks of gestation. Studies have evidenced that the dura mater becomes apparent at 8–9 weeks[18]. The present study, demonstrates that at 12 weeks, the dura mater can be readily recognized and presents as a multilayer fibrous structure. At this stage of development, the dura mater appears to be relatively thin, and its constituent cells are primarily fibroblasts. At 13 weeks of development, the dura mater appears thicker, and its cells are now mainly fibrocytes. From 12 to 13 weeks of maturation, the dura mater appears attached to the posterior wall of the spinal canal, and to the PAAM. At the 14 week stage, the dura mater becomes detached from the posterior wall of the spinal canal with some fibrous formations connecting them within the posterior atlanto-axial interspace. Researchers suggest that active fetal movement is the cause of the secondary epidural space, with the lateral and posterior portions of the spinal dura being separated from the spinal canal[18]. During subsequent development, the dura mater gradually thickens and becomes denser, and the fibers connecting the dura mater to the posterior wall of the spinal canal also become denser. The atlanto-occipital and atlantoaxial interspaces have a unique tissue morphology, and there are dense fibrous connections between suboccipital muscles and dura mater. These fibrous formations have been referred to as the MDB in adults[1].

At the 12 week stage, myotubes can be readily observed in the suboccipital muscles(Fig. 1.a) with mature muscular ducts appearing[19]. During subsequent development, the myotubes undergo further cell fusion. Additionally, during this stage, there appears the expression of contractile proteins forming mature muscle fibers and developing into functioning muscles with contractile capabilities[20]. After 12 weeks, the myotubes appear to have differentiated into muscle fibers, representing the final stage in the differentiation of skeletal muscle. Through our morphological observations, the morphology of the muscles appears mature at 19 weeks (Fig. 2.a).

The MDB fibers are already in place at 12 weeks and continue to differentiate progressively within the connective tissue. At 12 to 13 weeks, the cells constituting the MDB are primarily fibroblasts, but the fibers themselves are disorderly arranged. From 14 weeks onward, although the cells of the MDB remain primarily fibroblasts, the arrangement of the fibers has now become more orderly and arranged in parallel. At 14 weeks, it can be observed that the dura is separated from the posterior wall of the spinal canal. Interestingly, it is known that fibroblasts form linear bundles along the direction of traction during development[21]. Therefore, the separation of the dura mater from the posterior wall of the spinal canal may provide tractional forces from the MDB and cause the fiber arrangement in the dura to become more orderly. At 21 weeks, the fibers of the MDB appear denser, and the cellular components of the MDB are now dominated by fibrocytes. Previous studies have shown that tension generated by the RCPmi muscle can cause the dura mater to move via the transmission of forces from the MDB[22]. The present study found that the MDB matures later than the suboccipital musculature. During week 25 through to 30 weeks, some of the MDB fibers were observed to be originating from the To Be Named Ligament (TBNL), which is a dense fibrous structure emanating from the posterior part of the nuchal ligament (NL)[8]. It was also observed that the MDB at the suboccipital muscle level is denser and more orderly than the MDB located at the TBNL level. Therefore, our results show that the MDB is stretched by the suboccipital musculature during its development.

Previous research has demonstrated that head movement helps to promote circulation of cerebrospinal fluid (CSF), and suggest that the MDB may transform tensional forces generated by the suboccipital muscles to assist in CSF flow[17]. During pregnancy, mothers can usually feel their babies movements in their uterus from 16 to 20 weeks[23]. In fact, fetal movement happens before the mother detects them because the movements are felt by the mother only when they are sufficiently strong enough to stimulate the abdominal wall[24]. Studies have found that the fetus has obvious head movement at 14 to 16 weeks of gestation[25]. This is probably related to the separation of the spinal dura from the spinal canal at 14 weeks in the present study. According to Shiota (1983), two mechanical factors can mobilize the dura mater: differential growth between the spinal cord, the vertebral column, and the dura mater, and fetal movements. At 17 to 19 weeks, the fetus's head moves more frequently[25] and the development of the suboccipital musculature is becomes fully mature, while the MDB becomes mature at 21 weeks. Therefore, we believe that head movement and muscle activation help to promote the development of the MDB.

The present study explored cell differentiation of the dura mater, the MDB, and the suboccipital musculature during the development and growth of the suboccipital region. The cells of the spinal dura mater matured at 13W. Whereas muscle cells matured at 19W. The MDB cells showed the morphology of fibrocytes at 21W. Therefore, the tractional forces generated by the suboccipital muscles appears to promote the maturation of the MDB. The results of our study lay a foundation for future research on the biomechanics and function of the MDB.

Materials And Methods

The present study was approved by the Biomedical Ethics Committee of Dalian Medical University. Written informed consent was obtained from the fetal parents involved in the present study in accordance with the regulations of the ethics committee.

30 human fetus specimens (crown-rump length [CRL] 60–365 mm; gestational age of 12 to 41 weeks were observed in this study. The specimens were donated by the Department of obstetrics and gynecology at Lvshun District Hospital.

Specimen preparation

The fresh fetal specimens were perfused with 10% formalin through the umbilical vein. After umbilical vein perfusion, the suboccipital region of the fetuses, including the occipital bone, atlas, axis, suboccipital muscles, spinal dura mater (SDM), and the spinal cord were removed and fixed using 10% formalin.

Histological slices and H&E staining

The samples were decalcified using Jiang Weizhong Decalcifying solution until the bones in the tissues could be pierced easily by a needle. After the decalcification procedure, the samples were washed with flowing water for 12 up to 24 hours. Regular dehydration, as well as transparency and paraffin embedding methods were utilized. The embedded tissue blocks were sectioned into 10μm thick slices using a rotary microtome (Leica Micro HM450; Leica Microsystems GmbH, Wetzlar, Germany). For analysis under the light microscope, the sections were stained with hematoxylin eosin stain (provided by Shanghai yuanye Bio-Technology Co., Ltd.).

Declarations

Data availability

All data generated or analyzed during this study are included in this article. All the data was obtained via the above experiments.

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Author Contributions

H.J.S.and J.F.Z. conceived and designed the experiments. Y.S., H.X.L., T.W.S. and J.G. performed the experiments. B.L. carried out formal analysis, investigation. Y.Y.C., C.Y., J.Z., S.Z.S., C.H.Z., W.T., N.F., W.H.Y., and Y.F.W. participated in the experiment implementation and the experimental management. Y.S and H.X.L conducted the analyses, prepared figures 1-3, and wrote the manuscript. H.J.S., J.F.Z. and H.G. reviewed the manuscript.

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Competing financial interests

The authors declare no competing financial interests.

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Figures

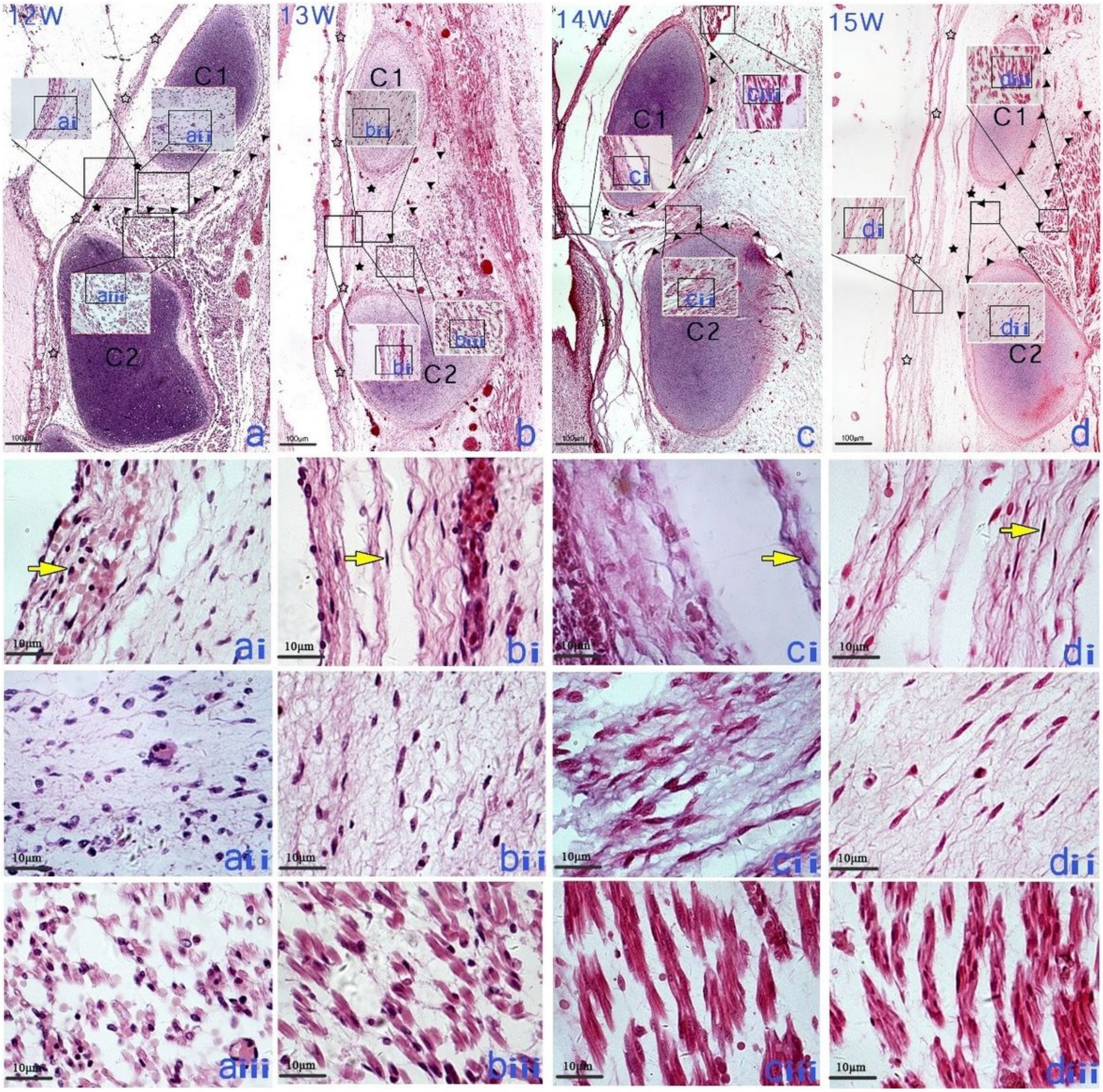

Figure 1

The posterior atlanto-axial interspace of the sagittal sections stained with HE stains at the 12 to 15 week human fetuses. The scale bars in images a-d are the same (4X), and those in images a-d i-iii are the same (40X). Images a-d i-iii are high-magnification views of the squares seen in images a-d. The multi-layered dura mater (hollow star) closely attaches to the posterior atlantoaxial membrane (filled star) (a, b). The dura thickened gradually over time and transformed from fibroblasts(a) to fibrocytes (b , c ,d) yellow arrow→). A gap appeared at the caudal end of the posterior atlantoaxial membrane and superior to the axis (C2), many obvious myodural bridge-like structures (MDB) (filled arrowhead▲)blended into the dura mater through the gap (c, d). The orientation of cells of the myodural bridge fibers are now parallel and more orderly arranged (a -d). At this stage of development, the myofibrils of the suboccipital muscles gradually increased and the number of nuclei decreased (a -d). Abbreviations: C1 = atlas; C2 = axis; MDB = myodural bridge.

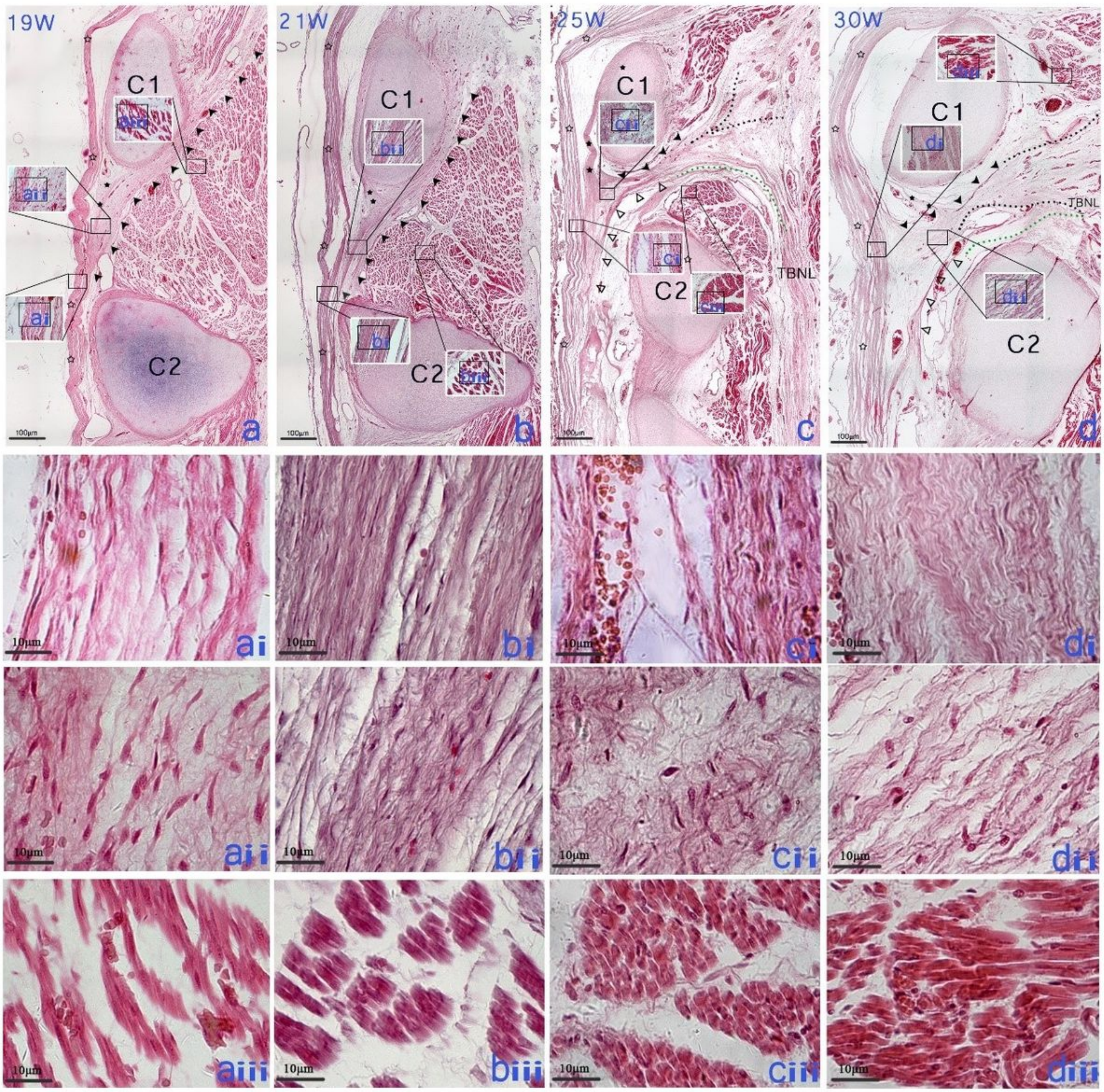

Figure 2

The H&E-stained sagittal sections from the posterior atlanto-axial interspace in human fetuses at 19, 21, 25, and 30 weeks. The scale bars in images a-d are the same (4X), and those in images a-d i-iii are the same (40X). Images a-d i-iii are high-magnification views of the squares seen in images a-d. The fibers of the MDB (filled arrowhead▲) pass through the posterior atlanto-axial membrane (PAAM) filled star within the posterior atlanto-axial interspace (a-d). (a, b): Fibers (filled arrowhead▲) travel longitudinally through the atlanto-axial interspace and extend downwards and forwards, eventually fusing with the SDM (hollow star). (c, d): Most of the dense fibers (filled arrowhead▲) originate from the suboccipital muscles, and a few fibers arise from the TBNL (hollow triangle △). The fibers of the dura mater are now significantly denser (a - d). The MDB cells are mainly fibroblasts a and gradually become fibrocytes (b). Fiber bundles tend to be oriented parallel to each other (b -d). The morphology of the muscles is relatively mature (a -d). Abbreviations: C1 = atlas; C2 = axis; SDM = spinal dura mater; TBNL= To Be Named Ligament; Black dotted line = direction of the fibers arising from the posterior occipital muscles; Green dotted line = direction of the fibers arising from the TBNL.

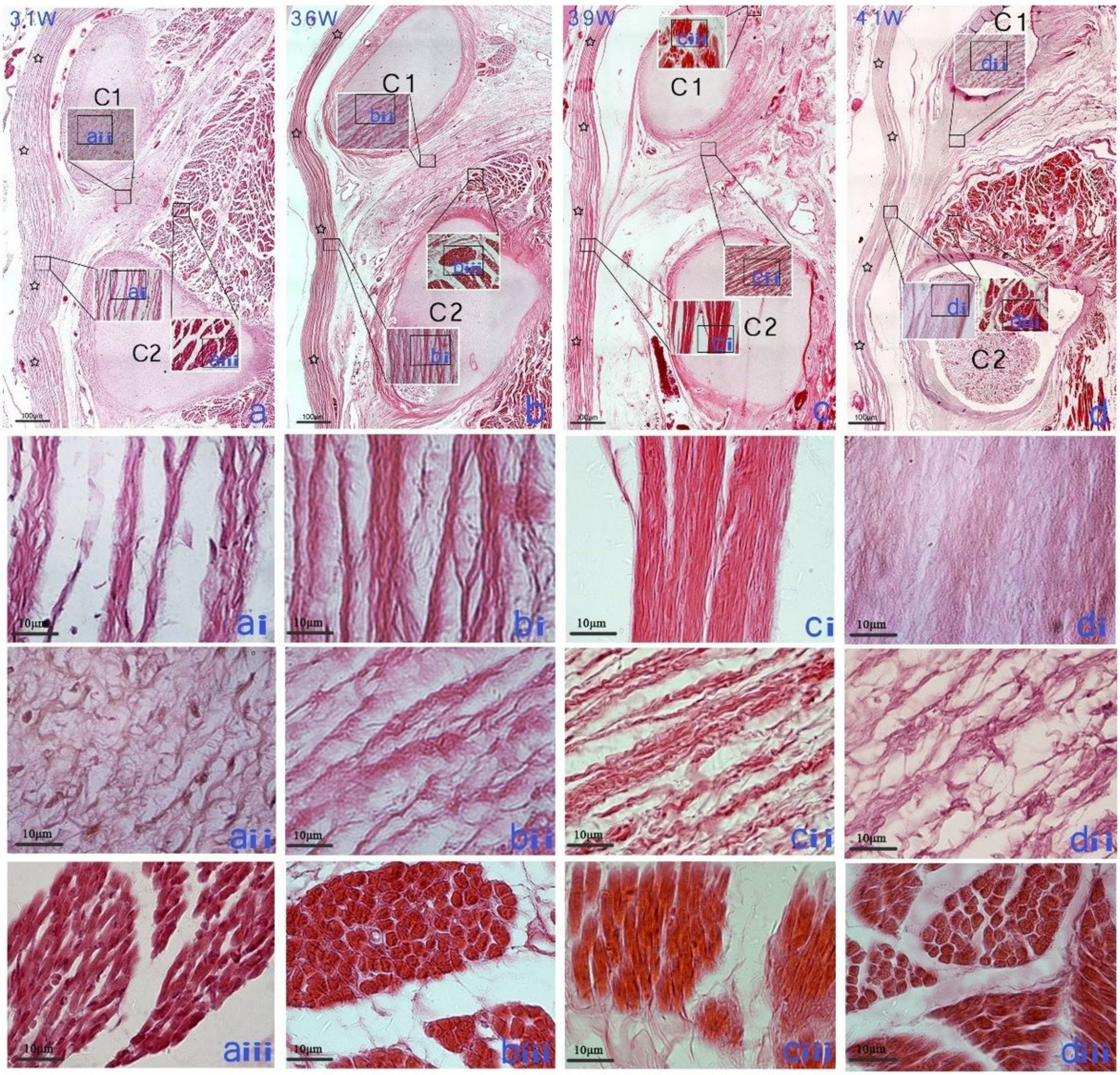

Figure 3

The H&E-stained sagittal sections of the posterior atlanto-axial interspace at 31, 36, 39, and 41 weeks old human fetuses. The scale bars in images a-d are the same (4X), and those in images a-d i-iii are the same (40X). Images a-d i-iii are high-magnification views of the squares seen in images a-d. The cells of the dura mater and MDB have essentially become fibroblasts, the fibers have become thicker and denser (a - d ,a -d). Muscle fibers have become more mature and fuller (a -d). Abbreviation: C1 = atlas; C2 = axis.