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ORIGINAL ARTICLE

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Comparative anatomy of the subclavius muscle and clavicle: a histological study using human, swine and mouse fetuses

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ABSTRACT

Background: Some mammals including the swine carry a fibrous vestigial clavicle, but a subclavius muscle (SBM) extends between the first rib and the supraspinatus muscle surface fascia. We aimed to examine development of the SBM and clavicle for finding a specific factor to provide the curious morphology.

Materials and methods: Histological sections of early- and midterm fetuses of the swine, human and mouse were observed and compared at the almost same morphological stage.

Results: In all three species, the initial SBM was seen extending between the cartilaginous first rib and a mesenchymal clavicle. At the early stage, the human and mouse fetuses carried the mesenchymal manubrium sterni above the heart bulbus as well as the acromion above the humeral head. However, in the swine fetus, the manubrium remained far caudal to the first rib, while the acromion was in the laterocaudal side of the glenohumeral joint. In place of the acromion, the swine supraspinatus muscle was large and covered the humeral head. At midterm, the human and mouse SBM attached to the membranous bone of the clavicle. Endochondral ossification occurred at the lateral and medial ends of the human clavicle, while it was seen in the medial half of the mouse clavicle anlage with a homogenous eosinophilic matrix.

Conclusions: The swine clavicle seemed to lose the endochondral parts due to the caudally-shifted manubrium sterni and acromion. The medial or clavicular attachment of the swine SBM might migrate to a nearby fascia of the supraspinatus muscle in the later development.

Keywords: subclavius muscle, clavicle, manubrium sterni, fetal development, swine, mouse, human

INTRODUCTION

A fact that membranous ossification occurs in the clavicle is known well, but the medial (sternal) and lateral (scapular) parts are formed with endochondral ossification [9]. In human fetuses, the medial end of the clavicle develops together with the manubrium sterni as a cartilage complex [13]. Generally in mammals, the pectoralis and cleido-occipitalis muscles sandwich the clavicle from the caudal and cranial sides, respectively, although the latter includes the sternocleidomastoideus muscle [4].

The clavicle is very small, vestigial or absent in some mammals such as carnivorans [2, 5, 12]. Likewise, the late-term swine fetus has a fibrous vestigial clavicle [6, 14] and the fact has recently become well-known since the large fetus is easy to obtain commercially (Fig. 1). The sternocleidomastoideus and pectoralis major muscles attach to the fibrous clavicle at the cranial and caudal margins, respectively. The swine cleidobrachialis muscle seems to correspond to the pars clavicularis of the human pectoralis major muscle (Fig. 1A, C), Notably, the swine also has a muscle named “subclavius” (Fig. 1B), but strangely, the muscle extends between the rib and a surface fascia of the supraspinatus muscle without attaching to the vestigial clavicle [4, 14]. Using histological sections of fetuses of the human, mouse and swine, the aim of this study was to compare development of the clavicle and subclavius muscle (SBM) for finding a specific factor to provide the curious morphology, “the SBM without clavicle”.

MATERIALS AND METHODS

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in 2013). We observed paraffin-embedded serial histological sections from 1) 15 early and midterm human fetuses, 2) 12 mouse early fetuses and 3) 6 swine fetuses. All sections were part of the large collection kept at the Department of Anatomy of the Universidad Complutense, Madrid, and the resulting embryos were obtained from miscarriages and ectopic pregnancies from the Department of Obstetrics of the University. The sectional planes were horizontal. The sections had been stained with hematoxylin and eosin (H&E) or azan. This study was approved by the Ethics Committees of Complutense University (B08/374).

In addition, for our learning and writing the Introduction, we dissected a swine late-term fetus (obtained commercially from TOKYOSHIBAURAZOUKI Co, Ltd, Tokyo, Japan), took photos of the pectoral girdle muscles (Nikon D7500, Nikon Corporation, Tokyo, Japan) and prepared routine histology after fixation with immersion into formalin solution (10% w/w) for 10 days.

RESULTS

Finding of a compatible stage of the pectoral girdle among the human, mouse and swine fetuses

We have a huge collection of human and mouse fetus histological sections, in contrast to a limited number of swine histological sections, Thus, first, we chose two sets of suitable swine sections for observations: a fetus with 9.1 mm CRL for horizontal sections (Fig. 2) and another with 9.0 mm for sagittal sections (Fig. 5). Next, we searched human and mouse sections showing a stage compatible with the swine pectoral girdle at the 9.0–9.1 mm stage. Accordingly, we chose two pairs of human and mouse fetuses in the huge collection: i) one pair was cut horizontally and a mouse fetus with 7 mm CRL (Fig. 3) and a human fetus with 21 mm CRL (Fig. 4); ii) another pair was cut sagittally and a mouse fetus with 6.9 mm CRL (Fig. 7A) and a human fetus with 17 mm CRL (Fig. 6). Finally, for comparison of ossification modes in the clavicle, we chose a pair of midterm fetuses: a mouse fetus with 15.5 mm CRL (Fig. 7C) and a human fetus with 125 mm CRL (Fig. 7E).

Comparison among horizontal sections of early fetuses

The developing manubrium sterni and clavicle were absent in swine sections above the heart bulbus (Fig. 2A–C) as well as in the level of the humeral head (Fig. 2D, E). Instead of the cartilaginous clavicle, a mesenchymal condensation was sandwiched by 1) the pectoralis major muscle and a head of the sternocleidomastoideus muscle (Fig.

2F) and 2) the other heads of the sternocleidomastoideus muscle and a candidate SBM. This SBM inserted to the cartilaginous first rib in the relatively lateral site (Fig. 2H). Instead of a bulky clavicle, multiple bundles of the sternocleidomastoideus occupied a large space in the lower cervical and pectoral regions between the humeral head and trachea.

In the human and mouse fetuses, the cartilaginous bulky clavicle, cartilaginous humeral head and mesenchymal manubrium sterni were together contained in a single horizontal section (Fig. 4C, D) or nearby sections (Fig. 6D, E) above the heart bulbus. The heart ventricles existed in the level of the scapular plate (Fig. 3H). Fibrous connections were not clear at the SBM attachment to the clavicle (Figs. 3D, 4G), but they were evident at the attachment to the cartilaginous rib: thus, the muscle insertion was histologically established first at the rib side (Figs. 3H, 4H). Consequently, the heart bulbus was lower than the manubrium sterni in the human and mouse fetuses, whereas the manubrium developed at a site caudal to the heart bulbus in the swine fetus.

Comparison among sagittal sections of early fetuses

The cartilaginous scapular plate was much more laterally located in the swine fetus than the human fetus (Fig. 5, 6): thus, in humans, the medially-located SBM was seen in sections together with the craniocaudally extending scapular plate. The mouse scapular plate was also located in the far lateral side of the SBM (not shown). In the swine and human, the brachial plexus extended from a site above the SBM to a space sandwiched by the scapular plate and rib cage. In place of the caudally-located acromion, the swine supraspinatus muscle was much thicker than the human fetus. A single section was likely to contain both clavicular (lateral) and costal (medial) attachments of the SBM in the swine fetus (Fig. 5A), but in the humans, the rib attachment was far medial to the clavicular attachment (Fig. 6E vs. Fig. 6A). Therefore, the human fetus appeared to have a “wide shoulder”, whereas the swine had a “narrow shoulder”.

Ossification of the clavicle and the subclavius muscle attachment

The mouse early clavicle (or an anlage of the clavicle) was characterized by the homogenous cartilage-like structure with an eosinophilic matrix, but the histology was clearly different from the real cartilage at the same stage (Fig. 7A, B). Notably, the pale-pink colored clavicle extended between the mesenchymal manubrium and cartilaginous acromion. Thus, the mouse clavicle started development evenly along the entire length. Later, the initial clavicle changed into a cartilage with endochondral ossification at the medial half (Fig. 7C, G), whereas membranous ossification simultaneously occurred at the lateral half of the eosinophilic anlage of the clavicle (Fig. 7G).

In contrast, the human cartilaginous clavicle appeared first at the medial and lateral ends and, at a long intermediate part, membranous ossification occurred later (Fig. 7D–F, H). Therefore, the human clavicle had the endochondral epiphysis and the membranous bone-made diaphysis and, both also accompanied the periosteal ossification. The human and mouse SBM attached to the membranous bone of the clavicle, not to the endochondral bone (Fig. 7G, H).

DISCUSSION

The present study demonstrated that, even in the swine fetus, the SBM had both costal and clavicular attachments although the vestigial clavicle corresponded to a mesenchymal condensation. The SBM did not attach to the cartilage but a membranous bone. Therefore, in the later prenatal stages, the clavicular attachment without hard tissues seemed to migrate along the outer surface of the supraspinatus muscle. A similar migration of the muscle attachment was reported to occur with a sliding of fascial attachments along the hard tissue [8, 17]. However, in the swine fetus, instead of the definite clavicle, the muscle fibers might catch the thick fascia on the nearby supraspinatus surface. This story was also an analogy of the development of the human digastricus muscle anterior belly that later catches the posterior belly tendon [10].

The topographical relation between the scapula and rib cage was almost same between the human and swine fetuses. Otherwise, the lower positioned manubrium sterni seemed to provide an essential difficulty in developing cartilaginous clavicle since the clavicle and intercostoclavicular mesenchyme (an initial manubrium) develop together as a complex [13]. Why does the swine sternum take a lower position in early fetuses? A topographical anatomy of the developing heart bulbus might connect to the lower-positioned sternum since the so-called cervical sinus pushes down the sternum [16]. Even in humans, the clavicle is underdeveloped with the anomalous manubrium [3, 15].

Membranous ossification requires a mechanical support: during the ossification, flat but dense mesenchymal tissues in the cranium are supported by the expanding brain to provide the parietal and frontal bones, the squamosal parts of the occipital and temporal bones and the ala major of the sphenoid [7, 17]. Likewise, the endoderm-derived nasal cavity is expanding and determine the shapes of bones around the cavity [18]. The membranous bone-derived, mid-portion of the clavicle seemed to require a mechanical support by the cartilaginous medial and lateral parts of the clavicle, but the latter was absent in the swine fetus because of the caudally-shifted manubrium and acromion. The endochondral clavicle might have a same cell lineage with the manubrium sterni and/or acromion.

Two strong muscles (the sternocleidomastoideus and pectoralis major muscles) sandwich the swine vestigial clavicle, but they seemed not to mechanically support the membranous ossification. Likewise, the temporalis muscle is usually very strong in mammals, but it may not be enough for the prenatal expansion of the sphenoid ala major and temporal bone squamosa. Rather than muscle contractions, a mechanical stress from the temporomandibular joint is likely to accelerate those flat bone expansion [1].

Finally, to our knowledge, there might be no comparative study to characterize a specific histology of the mouse initial clavicle. Similar to long bones of the extremity [11, 18], the human clavicle showed a difference between the epiphysis and diaphysis in the prenatal development. In contrast, the mouse initial clavicle was homogeneous along the entire length. Notably, however, in the long eosinophilic anlage of the mouse clavicle, both the endochondral and membranous ossifications occurred. Such a complexity is also reported in the human head membranous bone: the developing frontal bone contains islands of cartilage [18].

Overall, although the histogenesis was different between species, the clavicle was considered as a complex of the membranous and endochondral bones. The development seemed to require a mechanical support by (and perhaps a same cell lineage with) the manubrium sterni above the heart bulbus and/or the acromion above the glenohumeral joint.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The authors confirm that the data supporting the findings of this study is available within the article.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki. The use of the study specimens was approved by the Ethics Committee of Complutense University (B08/374).

Author contributions

Samuel Verdugo-López: conceptualization, formal analysis, methodology, writing — original draft. **Kei Kitamura:** conceptualization, data curation, formal analysis, writing — original draft. **Gen Murakami:** formal analysis, investigation, supervision, writing — original draft, writing — review and editing. **Jose Francisco Rodríguez-Vázquez:** data curation, investigation, visualization; writing — review and editing. **Hitoshi Yamamoto:** data curation, investigation, visualization; writing — review and editing.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Figure legend

Common abbreviation for figures

A, acromion; BP, brachial nerve plexus; CBM, cleidobrachialis muscle; 8CC, cricoid cartilage; CL, clavicle; DM, deltoideus muscle; E, esophagus; HB, hyoid body; HH, humeral head; IJV, internal jugular vein; ISM, Infraspinatus muscle ;LSM, levator scapulae muscle; LX, larynx; M, manubrium sterni; MC, Meckel's cartilage; MHM, mylohyoideus muscle; OHM, omohyoideus muscle; PMM, pectoralis major muscle; PX, pharynx; R1, first rib; S, scapula; SBM, subclavius muscle; SCM (SCM1, SCM2 and SCM3), sternocleidomastoideus muscles; SCV, subclavian vein; SHM, sternohyoideus muscle; SSM, supraspinatus muscle; STM, sternothyroideus muscle; TC, thyroid cartilage; THM, thyrohyoideus muscle; TM, trapezius muscle; TR, trachea.

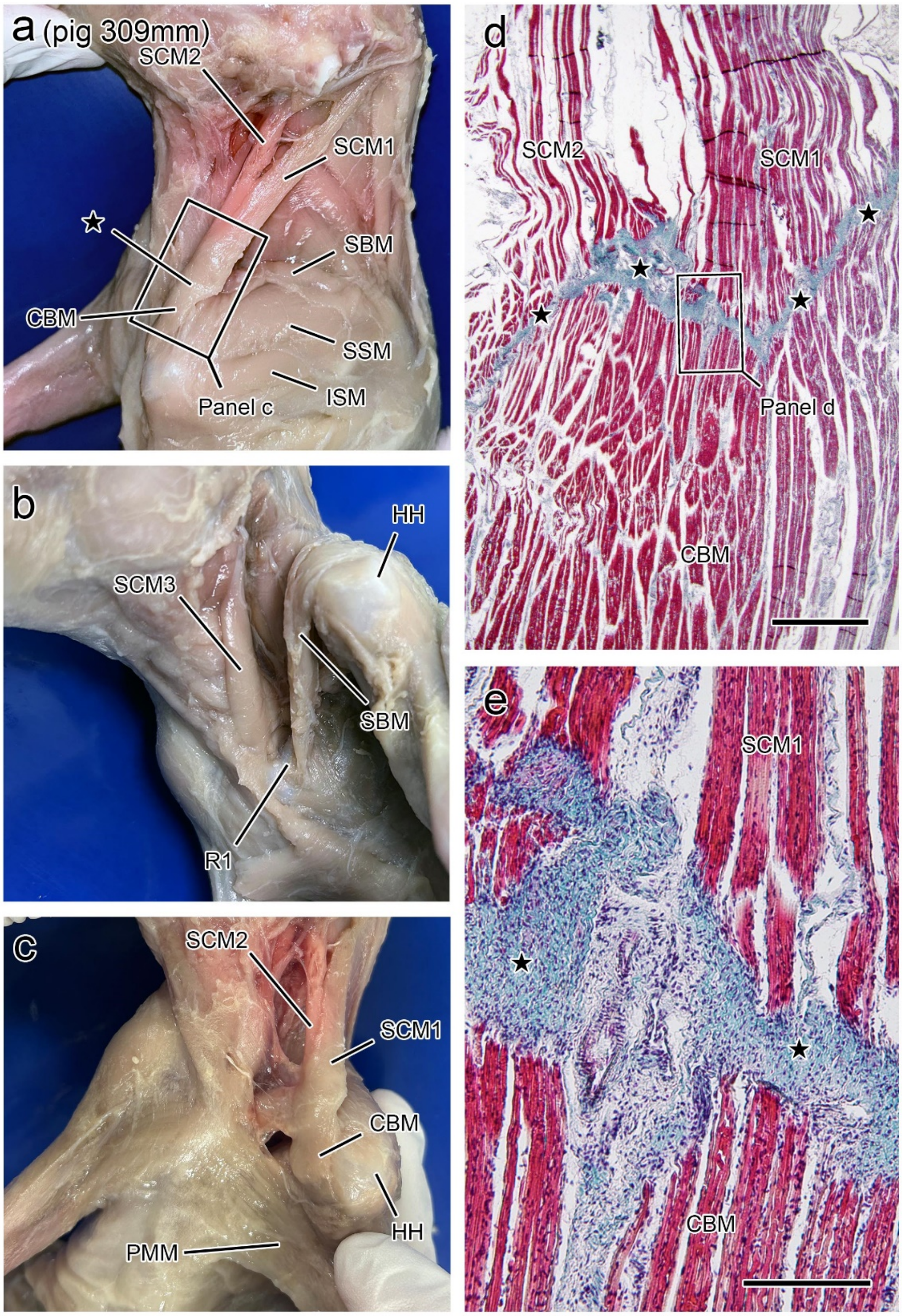

Figure 1. Gross anatomy of swine pectoral girdle muscles and histology of their attachments

to the vestigial clavicle. Left girdle of a late-term fetus (309-mm CRL). **A.** Upper left-sided view; **B.** Lower left-sided view; **C.** Anterior left-sided view. In panel A, the lower attachment of two sternocleidomastoideus muscles (SCM1 and SCM2) and the upper attachment of the cleidobrachialis muscle (CBM) sandwich the vestigial clavicle (star). Panel B displays the attachment of the subclavius muscle (SBM) to the first rib (R1). The cleidobrachialis muscle is regarded as a superficial, upper and posterior division of the pectoralis major muscle (**C**). Panel d exhibits histology of the vestigial clavicle and a square in the panel is shown in panel **E**. The vestigial clavicle is composed of fibrous tissues without cartilage. Therefore, the sternocleidomastoideus and cleidobrachialis muscles appeared to be a digastric muscle. Magnification (scale bars: 1 mm in panel D; 0.1 mm in panel E). Other abbreviations, see the common abbreviation.

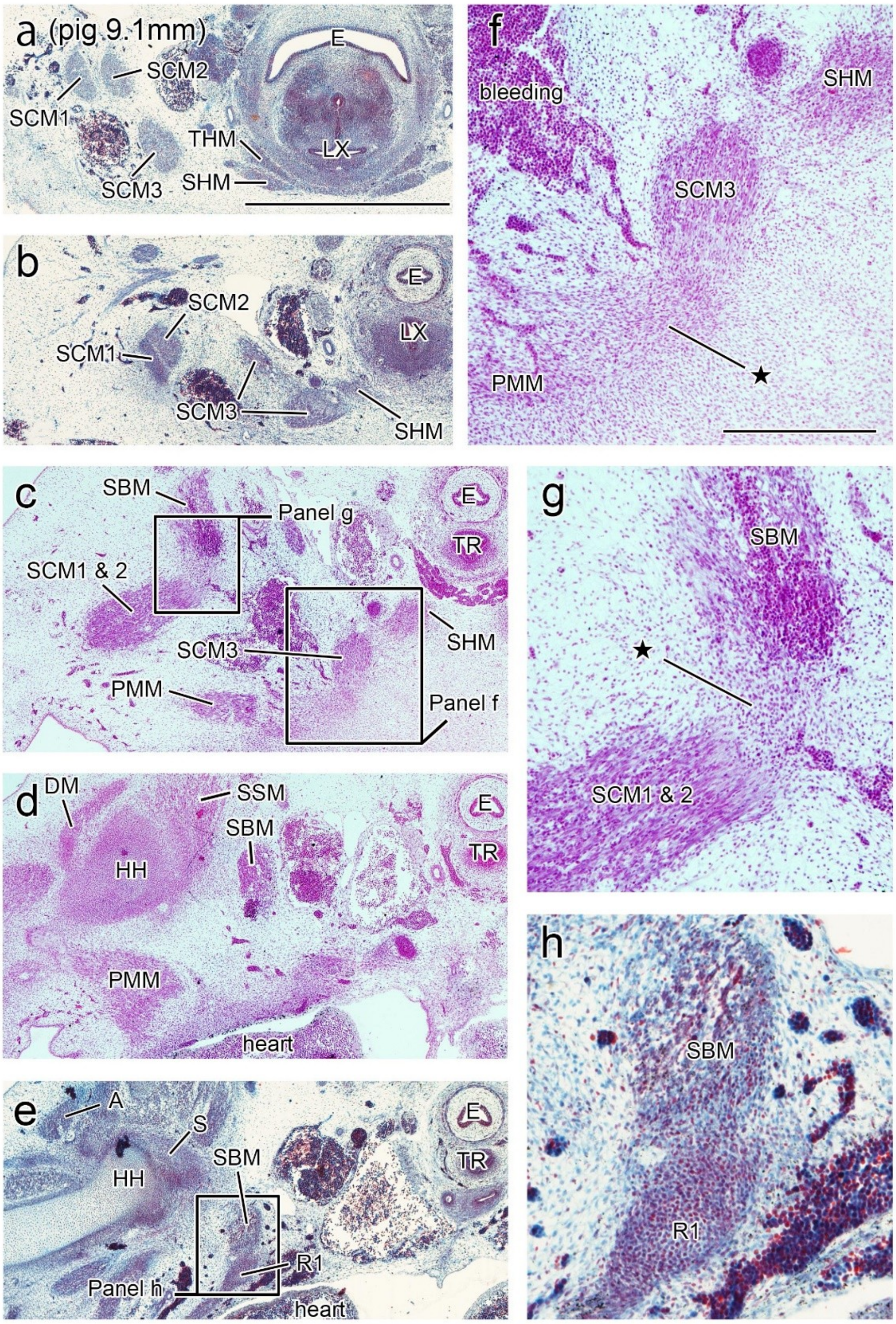

Figure 2. Swine horizontal sections containing no clavicle and manubrium sterni. A fetus with

9.1 mm CRL. Azan staining (A, B, E, H) and H&E staining (panels C, D, F, G). Panel a is 0.5 mm superior to panel e. Squares in panels c and e are shown in panels F–H at the higher magnification. At the lower cervical level, the sternocleidomastoid muscles are divided into three bundles (SCM1, 2 and 3 in panels A–C). Notably, at the upper thoracic level, the manubrium sterni and clavicle are not seen and the acromion is very small (**D, E**). The SCM3 and pectoralis major muscle (PMM) sandwich a mesenchymal condensation (vestigial clavicle; star in **F**). Although at the different aspects, a complex of the SCM1 and SCM2 as well as the subclavius muscle candidate (SBM) also attaches to the vestigial clavicle (star in **G**). Therefore, the subclavius muscle candidate (SBM) appears to originate from the mesenchymal condensation (**G**) and inserts to the cartilaginous first rib (R1; **E, H**). Panels A–E or panels F–H were prepared at the same magnification (scale bars: 1 mm in panel A; 0.1 mm in F). Other abbreviations, see the common abbreviation.

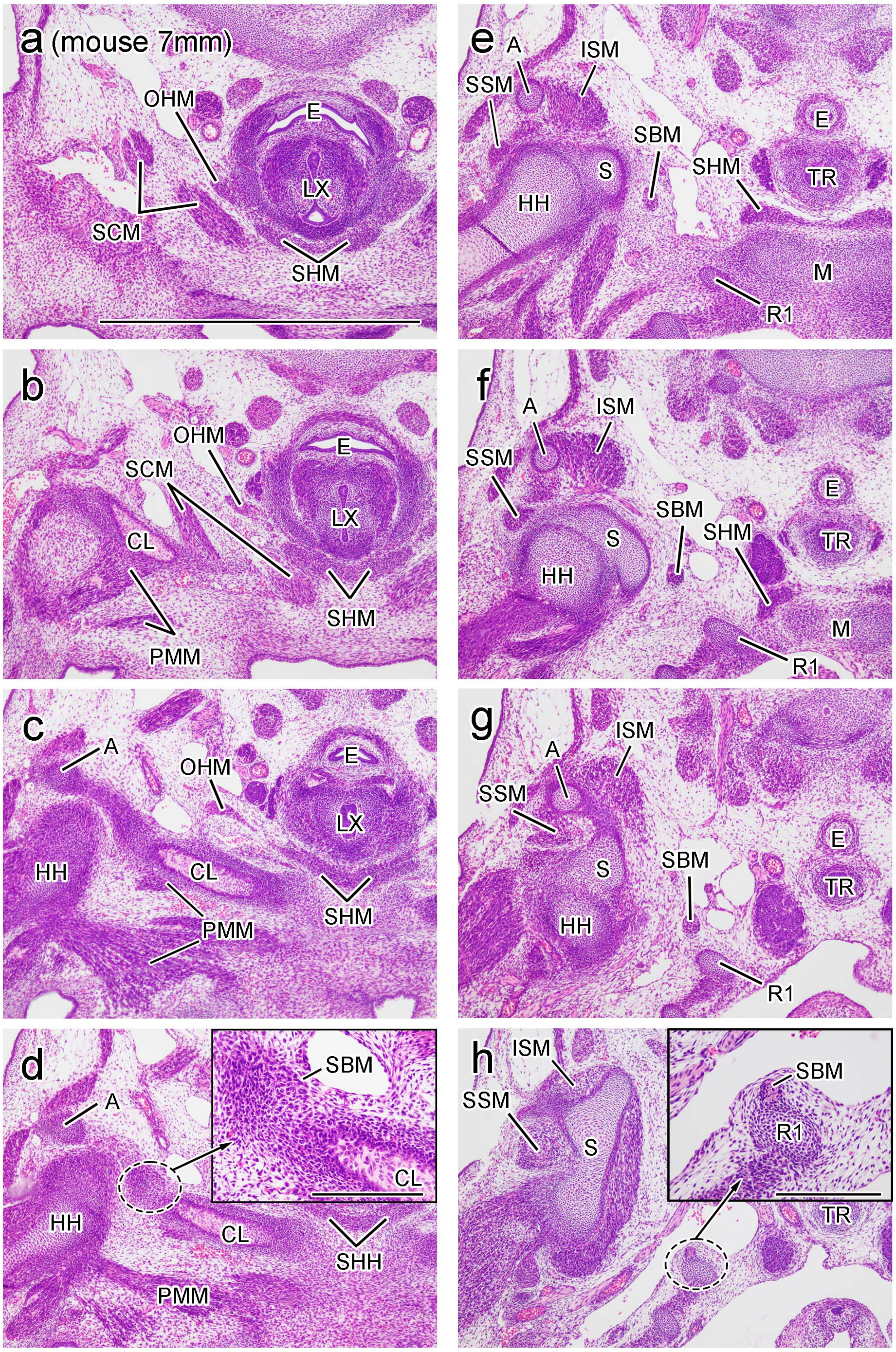

Figure 3. Mouse horizontal sections containing a long undifferentiated clavicle corresponding

to the medial half of the future clavicle.

A fetus with 7 mm CRL. HE staining. Panel a is 0.5 mm superior to panel h. Dotted line circle in panels d and h are shown in the inserts of the same panel at the higher magnification. The subclavius muscle (SBM) appears to originate from the lateral end of the pale-colored undifferentiated clavicle (panel D and its insert), runs inferiorly and provide a definite insertion to the cartilaginous first rib (R1; panel H and its insert). The manubrium sterni (M) and acromion (A) are seen in the inferior side of the undifferentiated clavicle (**E, F**). The lateral and medial ends of the clavicle are underdeveloped. Panels A–F or panels F–H were prepared at the same magnification (scale bars: 1 mm in panel A; 0.1 mm in two inserts). Other abbreviations, see the common abbreviation.

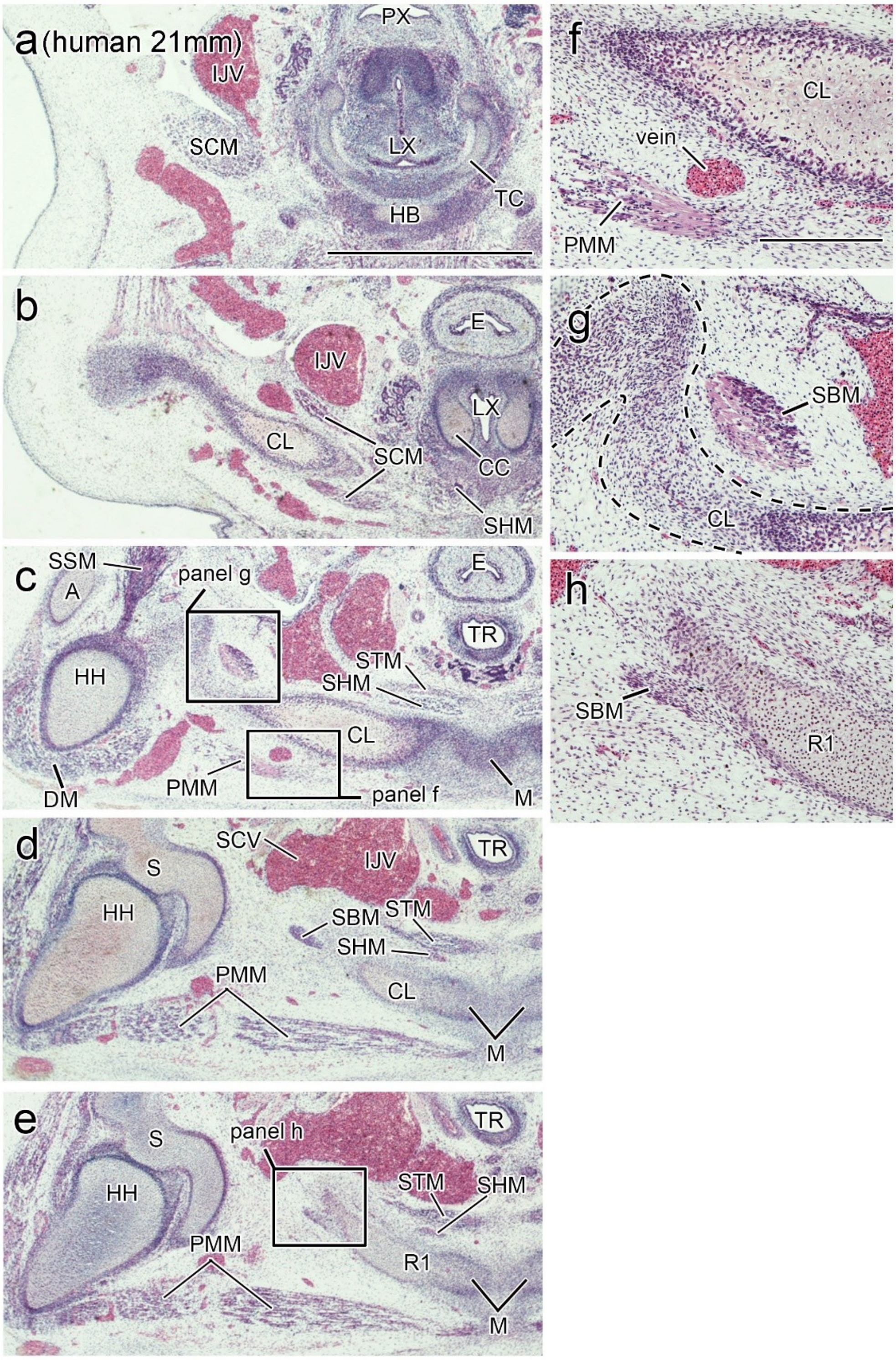

Figure 4. Human horizontal sections containing a long cartilaginous clavicle.

A fetus with 21-mm CRL. HE staining. Panel A is 1.6 mm superior to panel E. **F–H.** Higher-magnification views of squares (**C, E**). The long middle part of the clavicle appears to cartilaginous (**F**), but the lateral and medial parts remain mesenchymal (**C, G**). The manubrium sterni is mesenchymal at the upper part (**C, D**) and cartilaginous at the lower part attaching to the first rib (R1; **E**). The subclavius muscle (SBM) extends from the mesenchymal lateral part of the clavicle (**G**) to the cartilaginous rib (**H**). Panels A–E or panels F–H were prepared at the same magnification (scale bars: 1 mm in panel A; 0.1 mm in Panel F). Other abbreviations, see the common abbreviation.

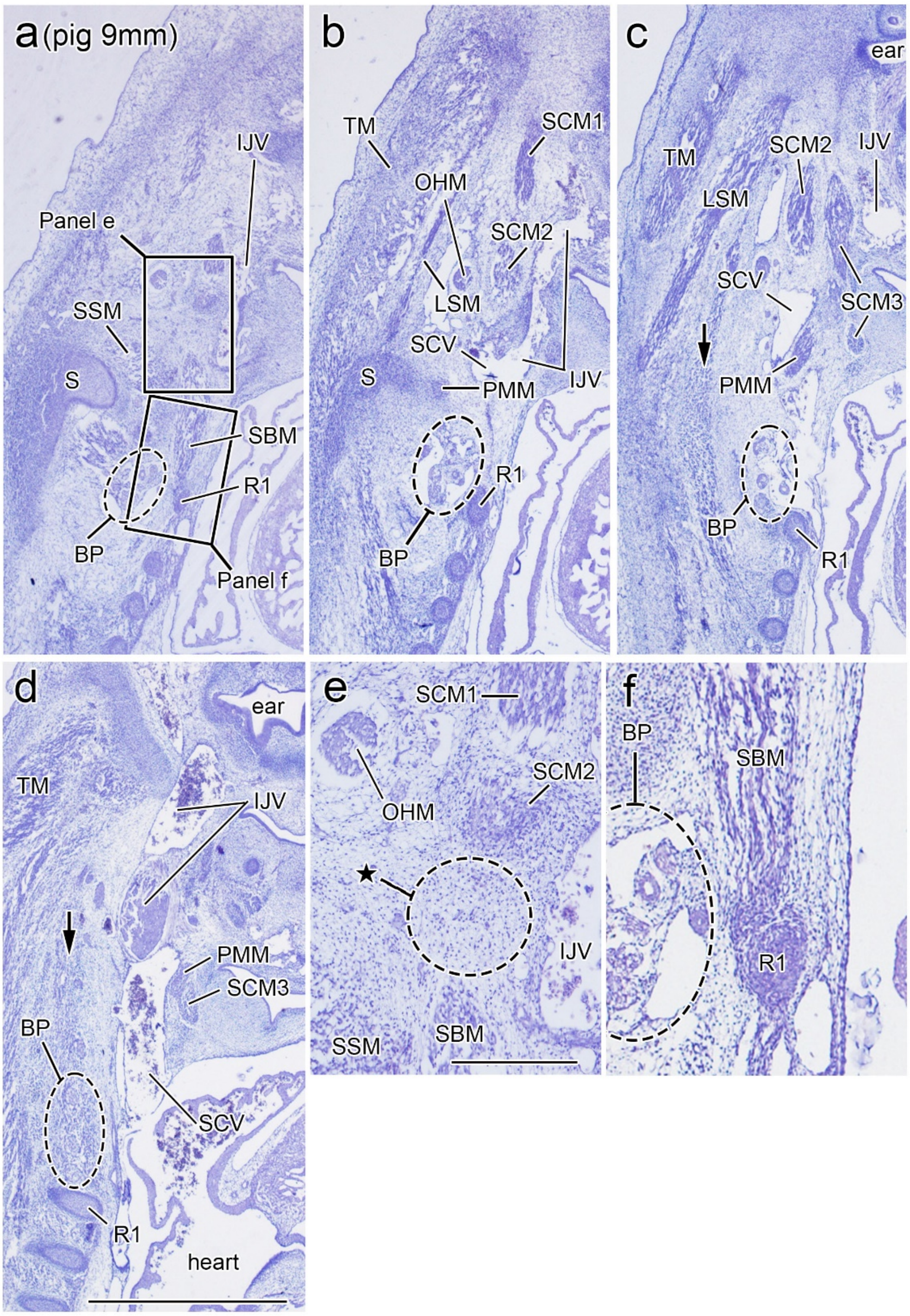

Figure 5. Swine sagittal sections without a scapular plate.

A fetus with 9-mm CRL. HE staining. Panel A is 0.3 mm lateral to panel D. Panels e and f are higher-magnification views of the squares in panel A. The subclavius muscle (SBM) originates from the first rib (**F**) and runs superiorly (**A**), but there is no mesenchymal condensation between the sternocleidomastoideus and subclavius muscles (circle with star in **E**). Arrow (**C, D**) indicates a thick muscle sheet possibly corresponding to the serratus anterior. Panels A–D or panels E, F were prepared at the same magnification (scale bars: 1 mm in panel A; 0.1 mm in panel E). Other abbreviations, see the common abbreviation.

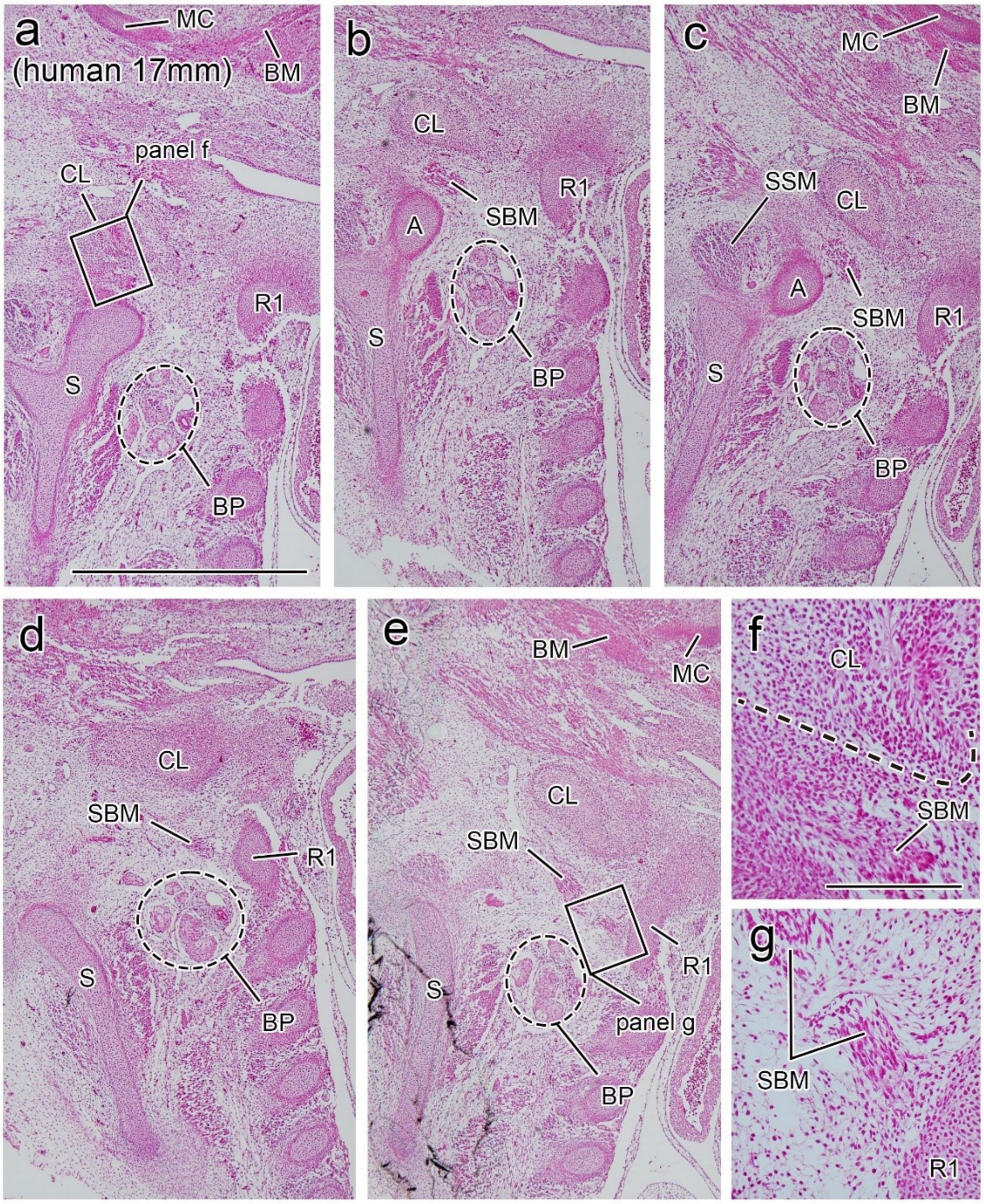

Figure. 6. Human sagittal sections with a cranio-caudally extending scapular plate.

A fetus with a CRL of 17 mm. H&E staining. Panel A is 0.4 mm lateral to panel D. **F, G.** Higher-magnification views of the squares in Panels A, E, respectively. The subclavian muscle (SBM) attaches to the clavicle (CL) near the acromion (A) at the lateral end (A, F), while it attaches to the posterior surface of the first rib (R1) (E, G). Panels A–E or panels F, G were prepared at the same magnification (scale bars: 1 mm in panel A; 0.1 mm in panel F). Other abbreviations, see the common abbreviation.

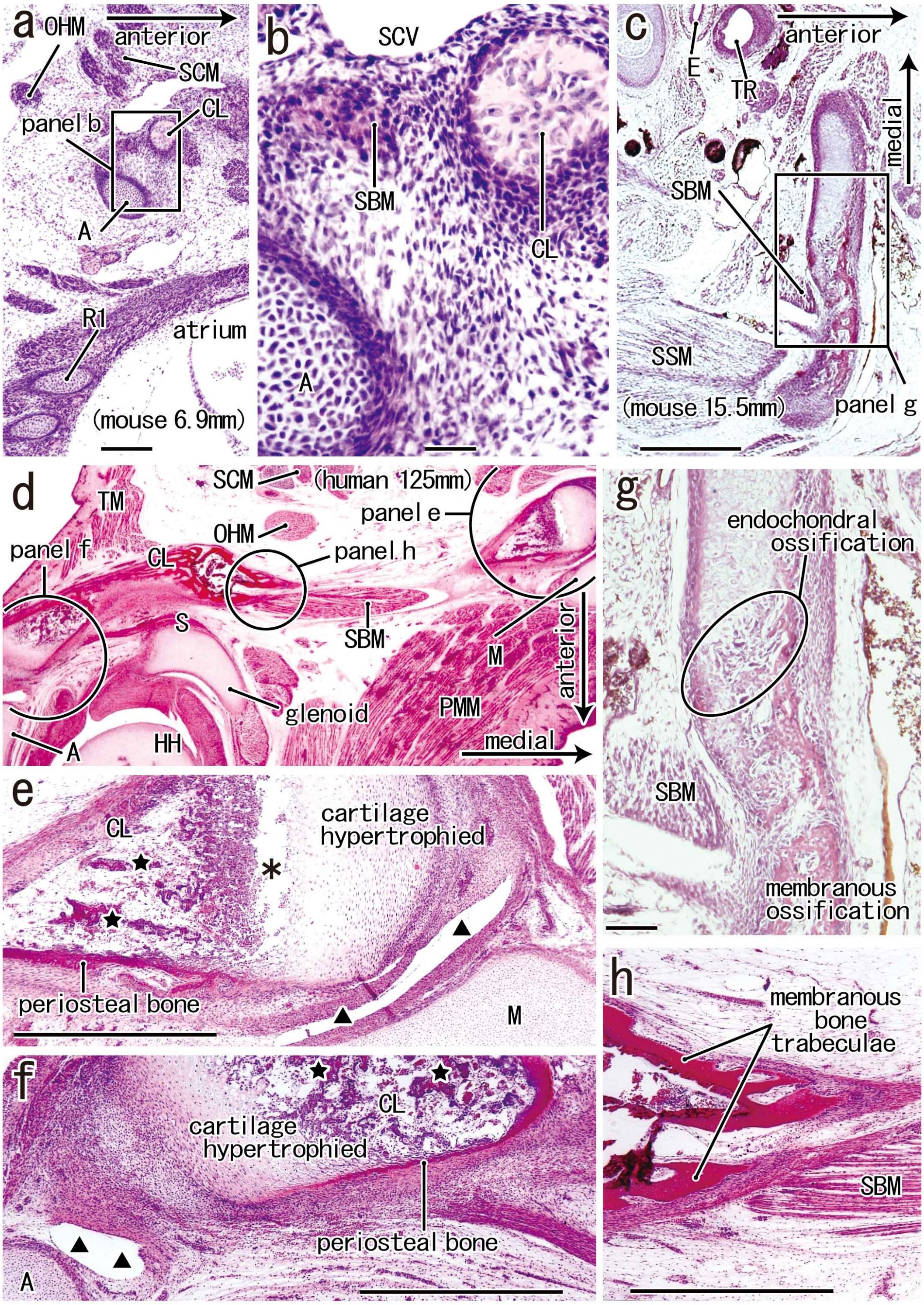

Figure 7. A combination of endochondral and membranous ossifications in the mouse and

human clavicles.

A, B. A sagittal section of a mouse fetus with 6.9 mm CRL; **C, G.** A horizontal section of a mouse fetus with 15.5-mm CRL; **D–F, H.** A horizontal section of a human fetus with 125-mm CRL. **B.** A higher magnification view of a square in panel A. The early clavicle of the mouse is characterized by the eosinophilic matrix that different from the cartilage (*i.e.*, A or acromion in panel b). The subclavius muscle (SBM) appears to attach to both the acromion and clavicle. Both membranous and endochondral ossifications are seen in the midterm mouse clavicle (C; the higher magnification view — G). **D.** The human midterm pectoral region that contains the clavicle articulating the manubrium sterni (M) and acromion (A). Endochondral ossification is seen at the medial end (E) as well as the lateral end (F), while membranous ossification occurs at the long intermediate part (D, H). Stars indicate endochondral bone trabeculae. Triangles indicate a joint cavity. Both in the human and mouse, the subclavius muscle attaches to a periosteum of the membranous bone (G, H). Asterisk in panel e indicates a tissue injury during the histological procedure. Scale bars: 1 mm in A, C, D, F–H and h; 0.1 mm in B, G). Other abbreviations, see the common abbreviation.