Received: 10 November 2019 | Revised: 17 February 2020 | Accepted: 18 February 2020

DOI: 10.1111/joa.13181

ORIGINAL ARTICLE

ANATOMICAL SOCIETY Journal of Anatomy WILEY

The incudopetrosal joint of the human middle ear: a transient morphology in fetuses

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Abstract

In spite of the amount of research on fetal development of the human middle ear and ear ossicles, there has been no report showing a joint between the short limb of incus and the otic capsule or petrous part of the temporal bone. According to observations of serial histological sections from 65 embryos and fetuses at 7–17 weeks of development, the incudopetrosal joint exhibited a developmental sequence similar to the other joints of ossicles, with an appearance of an interzone followed by a trilaminar configuration at 7–12 weeks, a joint cavitation at 13–15 weeks and development of intraarticular and capsular ligaments at 16–17 weeks. These processes occurred at the same time or slightly later than any other joint. Thus, the joint development might coordinate with vibrating ossicles *in utero*. The growing short limb of incus appeared to accelerate an expansion of the epitympanic recess of the tympanic cavity. Additional observations of five late-stage fetuses demonstrated the incudopetrosal joint located in the fossa incudis joint changing to syndesmosis. Consequently, a real joint with a cavity existed transiently between the human neurocranium and the first pharyngeal arch derivative (i.e. incus) in contrast to the tympanostapedial joint or syndesmosis between the neurocranium and the second arch derivative. The newly described joint might have an effect on the widely accepted primary jaw concept: the mammalian jaw should thus have been created within the first pharyngeal arch, although the connection with neurocranium by the stapes is of a different origin.

KEYWORDS

human fetus, incudopetrosal joint, incus, middle ear, otic capsule

1 | INTRODUCTION

The theory that the human incus has a 'dual origin' (i.e. is derived from both the first and second pharyngeal arches) was proposed by Anson and colleagues in the early 1960s (Hanson *et al.*, 1959, 1962; Anson *et al.*, 1960; Strickland *et al.*, 1962) and subsequently cited widely in many textbooks, including Gray's Anatomy (Williams 1995; Standring 2005). More recently, however, on the basis of excellent 3D reconstructions, Burford and Mason (2016) have provided strong evidence that the human incus originates entirely from the first arch. We have also demonstrated that, in the first pharyngeal arch, the human incus anlage is separated from an independent, Meckel's cartilage-malleus complex (Rodríguez-Vázquez *et al.*, 2018). Louryan *et al.* (2018) recently demonstrated that ossicular condensations of the malleus and incus in mouse embryo arise from both first and second arches. Therefore, our present understanding of the human ear ossicles is that the incus-otic capsule border in the epitympanic recess (see below) is simply an attachment of the first pharyngeal arch to the initial chondrocranium. As the Reichert's cartilage of the second arch does not provide a transient articulation to the otic capsule

(Rodríguez-Vázquez 2005, 2009), does the first pharyngeal arch derivative or the incus provide an articulation to the otic capsule or neurocranium?

During our recent studies of the fetal middle ear (Rodríguez-Vázquez *et al*., 2017, 2018), we incidentally found a joint with a cavity between the short limb of the incus and the posterior part of otic capsule or the petrous part of temporal bone (i.e. incudopetrosal joint). Although there have been numerous studies of fetal development of the ear ossicles (Anson *et al*., 1960; Hanson *et al*., 1962; Hough, 1963; Masuda *et al*., 1978; Cousins and Milton, 1988; Nomura *et al*., 1988; Ars, 1989; Rodríguez-Vazquez et al. 1991, 2011; Louryan, 1993; Takeda *et al*., 1996; Rodríguez-Vazquez, 2005, 2009; Whyte *et al*., 2009; Ozeki-Satoh *et al*., 2016), joints between ossicles (Masuda *et al*., 1978; Ars, 1989; Castellote *et al*., 1997; Whyte et al., 2002) and the middle ear cavity (Hammar, 1902; Proctor, 1964; Aimi, 1971; Palva *et al*., 1996; Palva and Johsson, 1995; Palva and Ramsay, 1996), we have found no description of the incudopetrosal joint.

The middle ear space can be subdivided into the tympanic cavity proper opposite the tympanic membrane and the epitympanic recess above the level of the membrane, the latter containing the upper half of the malleus and most of the incus in Gray´s anatomy (Standring, 2005). A number of structures are lodged on and within the posterior wall of tympanic cavity, a site known clinically as the retrotympanum (Anson and Donaldson, 1973; Cheiţă *et al*., 2010). The posterior or mastoid wall (paries mastoideus) is wider above than at its lower part. The epitympanic recess occupies the upper part of the posterior wall from which the recess opens into the tympanic antrum (Anson and Donaldson, 1973). A boundary between the tympanic cavity proper and the epitympanic recess is delineated by a bony prominence of the facial canal medially and a 'fossa of the incus' posteriorly where the posterior ligament of incus is attached (Anson and Donaldson, 1973). We hypothesized that the epitympanic recess as well as the fossa of the incus enlarges in association with development of ear ossicles and their joints. Consequently, the aim of this study was to clarify fetal development of the incudopetrosal joint with special reference to the enlarging epitympanic recess.

2 | MATERIALS AND METHODS

The study complies with the provisions of the Declaration of Helsinki (World Medical Association, 2013). A total of 65 embryos and fetuses from the collection of the Embryology Institute at the University Complutense of Madrid (Spain) were studied. The greatest length (GL) of the embryos ranged from 19 to 31 mm (Carnegie Stages [CS] 20–23; 7–8 weeks of development [WD]). For the fetuses, the GL ranged from 33 to 150 mm; 9–17 WD. Additional observations were conducted using semiserial sections from five late-stage fetuses (240–340 mm GL; 25–34 WD).The parameters used to determine postconceptional age were the GL and external and internal criteria (O'Rahilly and Muller, 2010) (Table 1).

All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material indicated possible malformation. Approval for the study was granted by the ethics committee of the university (B-08/374). All specimens were fixed in 10% neutral formalin and embedded in paraffin for processing. The sections ranged in thickness from 10 to 25 µm, depending on specimen size. The sections were stained with hematoxylin and eosin (HE), azan, orange-fuchsin (OF) and Bielschowsky.

TABLE 1 Number of specimens and variations in the incudopetrosal joint

Phase	WD	CS	Number of specimens	Number of specimens that show variation	GL (mm)	Concrete example of Variation
a (Figure 1)	7	20	6	-	-	-
	7	21	7	1	23	initial interzone
b (Figure 2)	8	22	3	-	-	-
	8	23	6	2	30–31	initial interzone
c (Figure 3)	9	-	9	-	-	-
d (Figure 4)	10	-	4	1	62	trilaminar interzone
	11	-	3	-	-	-
	12	-	4	2	72–76	homogeneous interzone
e (Figure 5)	13	-	10	1	85.5	trilaminar interzone
	14	-	6	1	110	syndesmosis
	15	-	3	1	137	syndesmosis
f (Figures 6-8)	16	-	2	-	-	-
	17	-	2	-	-	-
	25–34	-	5	-	-	-

Note: [a]Precartilaginous union of incus-otic capsule. [b] Cartilaginous union of incus-otic capsule [c] Homogeneous interzone. [d] Trilaminar interzone [e] Cavitation. [f] Syndesmosis. CS, Carnegie stages; GL, greatest length; IP, incudopetrosal joint; WD, weeks of development.

3 | RESULTS

3.1 | Precartilaginous union incus-otic capsule

At 7 WD (CS 20–21) a lateral protrusion in the otic capsule appeared. A precartilaginous connection was distinguishable between the short limb of the incus and the lateral protrusion of the otic capsule (a future petrous part of the temporal bone). In the area of union between the incus and otic capsule, continuity of the precartilaginous cells and the initial perichondrium was observed (Figure 1a-f). This connection was identified in several embryos at the same stage, being located lateral to the horizontal course of the facial nerve and

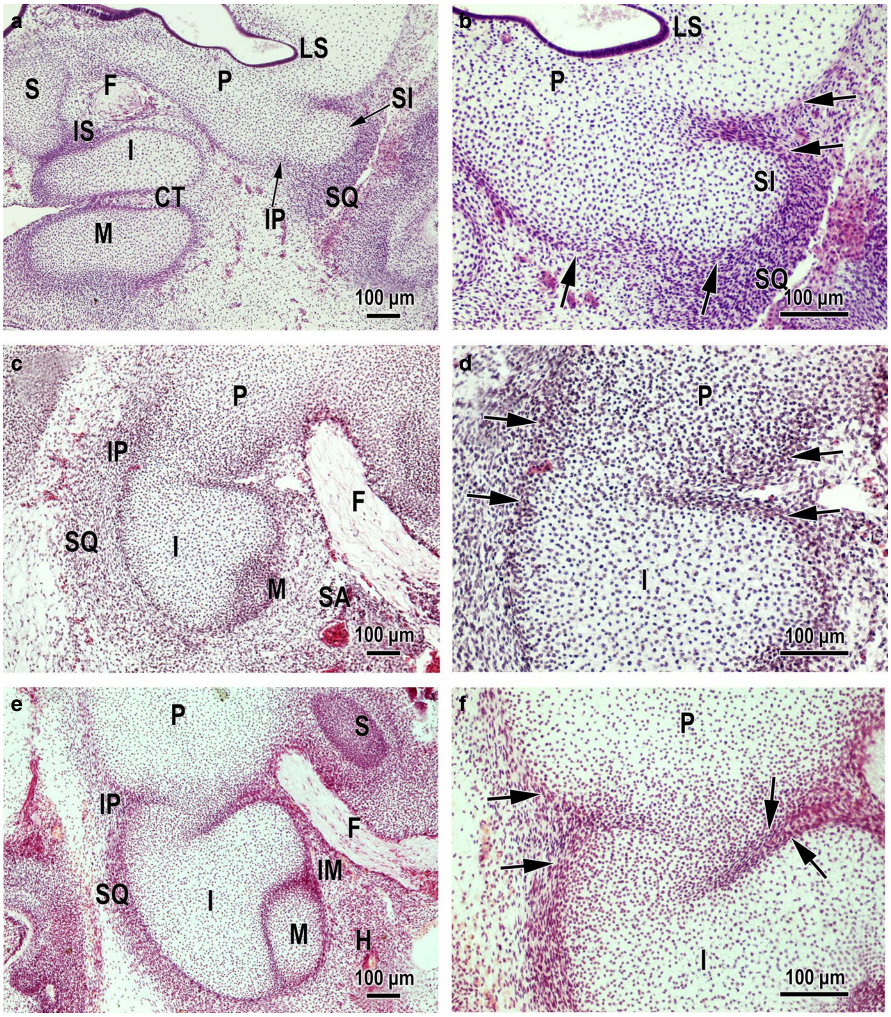

FIGURE 1 Precartilaginous union of incus-otic capsule. (a,b) 7 WD. Human embryo of 20 mm GL (20 CS). Frontal section. HE staining. (b) Enlargement of (a). Lateral to the facial nerve (F) and close to the semicircular duct (LS), a precartilaginous connection: union of incus-otic capsule (IP) is distinguishable between the short limb of the incus (SI) and the lateral protrusion of the otic capsule (P). The initial perichondrium (arrows) and precartilaginous cells are continuous in the union of the incus-otic capsule. (c,d) 7 WD. Human embryo of 23 mm GL (21 CS). Horizontal section. HE staining. (d) Enlargement of (c). The union between the incus and otic capsule (IP) is characterized by the continuity of the precartilaginous cells and the primordium of perichondrium (arrows). (e,f) 7 WD. Human embryo of 23.5 mm GL (21 CS). Horizontal section. HE staining. (f) Enlargement of (e). Topographical anatomy of the incus (I) and malleus (M). Union between the incus and otic capsule (IP) and the incudomalleolar joint (IM) was observed. In the incus-otic capsule union, precartilaginous cells and perichondral sheath (arrows) are continuous. Lateral to the incus, a mesenchymal condensation was evident, being the origin of the squamous part of the temporal bone (SQ). For other abbreviations, see common abbreviations below. AP, articular apophysis; AS, articular surface; BI, body of incus; C, articular capsule of the incudopetrosal joint; CA, articular cavity of the incudopetrosal joint; CT, chorda tympani nerve; D, disk of the temporomandibular joint; DM, discomalleolar ligament; E, epitympanic recess; EE, external ear; F, facial nerve; FI, fossa incudis; G, gonial/anterior process of malleus; GG, geniculate ganglion; H, hyoid artery; I, incus; IE, internal ear; IL, intraarticular/interosseous ligament; IM, incudomalleolar joint; IS, incudostapedial joint; IP, incudopetrosal joint/ union incus-otic capsule; LL, long limb of incus; LS, lateral semicircular canal; M, malleus; MK, Meckel´s cartilage; P, petrous part of temporal bone/ otic capsule; PG, parotid gland; PL, posterior ligament of incus; PS, petrosquamosal fissure; R, Reichert´s cartilage; S, stapes; SA, stapedial artery; SM, stapedial muscle; SQ, squamous part of temporal bone; SI, short limb of incus; SV, synovial of the incudopetrosal joint; TB, tympanic bone; TM, temporalis muscle; TS, tympanostapedial joint; TT, tegmen tympani

caudal to the lateral semicircular duct. Lateral to the incus, a mesenchymal condensation of the squamous part of the temporal bone appeared (Figure 1a,c,e). The short limb of the incus continued with the otic capsule at the level of the future posterior wall of the tympanic cavity. Therefore, the organization of the posterior and lateral wall of the tympanic cavity started at the future epitympanic recess. At CS 21, the future incudomalleolar joint was identified as an interzone phase between the body of the incus and the head of the malleus, whereas the incudostapedial joint was recognized as a homogeneous interzone.

3.2 | Cartilaginous union between the incus and otic capsule with the anlage of the fossa incudis

At the stage of 8 WD (CS 22–23), a cartilaginous connection was observed between the incus and the otic capsule. A continuity of cartilaginous cells and the perichondral sheath was located in the incus-otic capsule union (Figure 2b,d). In the interzone of the incudomalleolar joint, a small cavity was identified. The distance between the incus and the otic capsule was reduced due to the growth of both structures, resulting in development of the medial wall of the future fossa incudis (Figure 2a,c). The lateral wall of the epitympanic recess had not yet formed, since there was a condensed mesenchyme corresponding to the future squamous part of the temporal bone on the lateral wall of the fossa incudis (Figure 2a,c).

The end of the short limb of the incus was surrounded medially and inferiorly by the petrous part and laterally by the anlage of the squamous part of the temporal bone. A small cavity appeared in the posterior wall of the tympanic cavity, i.e. the future fossa incudis, where the end of the short limb of the incus was located (Figure 2e,f). The cavity of the incudomalleolar joint became larger and extended caudally, and cavitation in the incudostapedial joint became apparent.

3.3 | Homogeneous interzone phase of the incudopetrosal joint in the fossa incudis

At 9 WD, the homogeneous interzone in the incudopetrosal joint became evident. This interzone was located between the dorsal end of the short limb of the incus and the otic capsule, lateral to the facial nerve (Figure 3a,b).

The intramembranous ossification of the squamous part of the temporal bone almost reached the cartilaginous petrous part near the

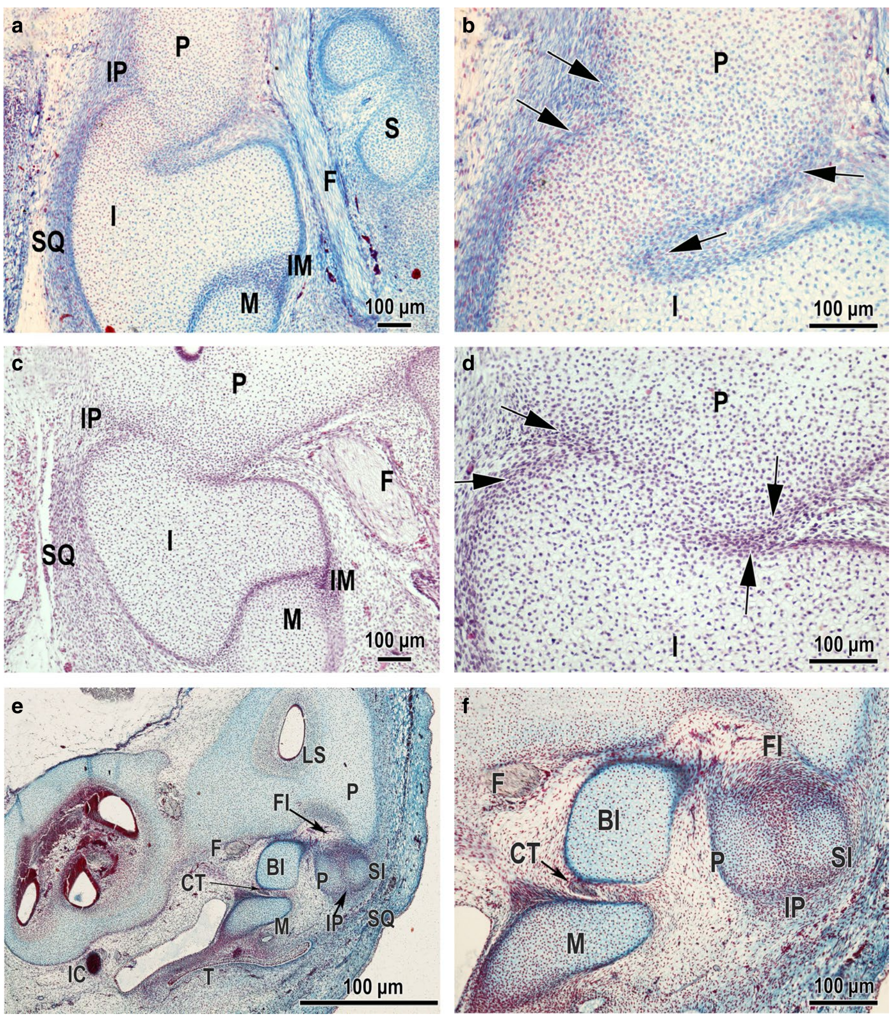

FIGURE 2 Cartilaginous union between the incus and otic capsule with the anlage of the fossa incudis. (a,b) 8 WD. Human embryo of 26.5 mm GL (22 CS). Horizontal section. Azan staining. (b) Enlargement of (a). There is a cartilaginous continuity in the incus-otic capsule (IP). The perichondral sheath of incus and otic capsule is continuous (arrows). (c,d) 8 WD. Human embryo of 29 mm GL (23 CS). Horizontal section. HE staining. (d) Enlargement of (c). A cartilaginous continuity in the incus-otic capsule (IP) and incudomalleolar joint (IM) is seen. The perichondral layer of incus and otic capsule is continuous (arrows) without interruption. (e,f) 8 WD. Human embryo of 28 mm GL (23 CS). Frontal section. Azan staining. (f) Enlargement of (e). The short limb of the incus (SI) is surrounded by the otic capsule (P; petrous part) and the anlage of the squamous part of the temporal bone (SQ). A small cavity appears in the posterior wall of the tympanic cavity, i.e. the future fossa incudis (FI). For other abbreviations, see Figure 1

lateral and posterior walls of the epitympanic recess. Notably, there was a small recess or cavity in the posterior wall of the epitympanic recess, i.e. the future fossa incudis (Figure 3a,b). The incudomalleolar joint carried a large articular cavity, and the tympanostapedial joint was in the interzone phase, similar to the incudopetrosal joint (Figure 3a).

3.4 | Establishment of the petrosquamosal fissure and closure of the posterior wall of the tympanic cavity. A trilaminar interzone phase of the incudopetrosal joint

At 10 WD, the petrosquamosal fissure had formed between the squamous part and the petrous part (otic capsule) of the temporal bone, although endochondral ossification of the otic capsule had not yet started. The posterior wall of the tympanic cavity was closed at the epitympanic recess and delimited by a small cavity or fossa incudis that received the end of the short limb of the incus on the dorsolateral side of the facial nerve (Figure 4a,b).

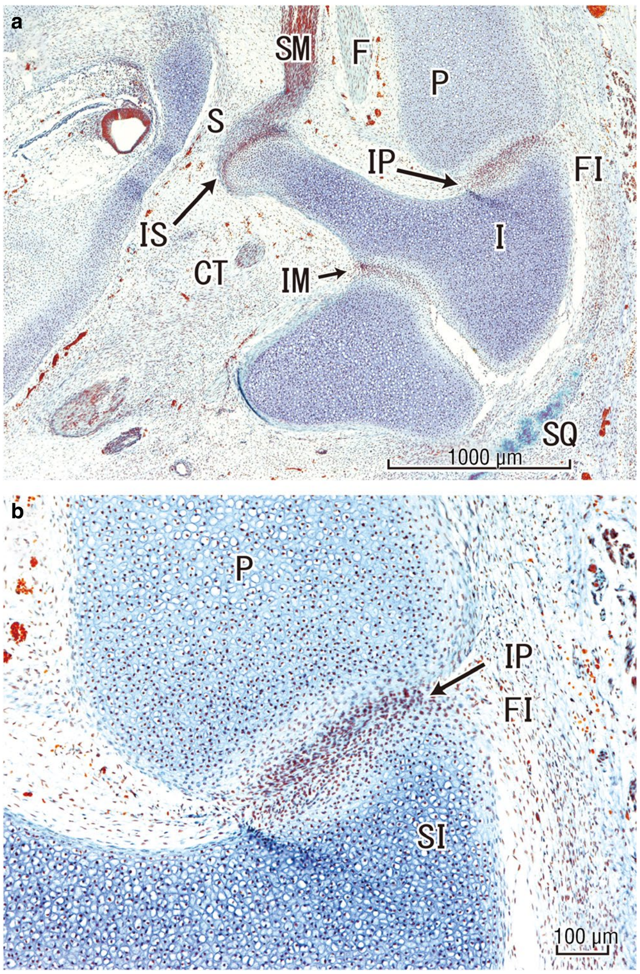

FIGURE 3 Homogeneous interzone phase of the incudopetrosal joint in the fossa incudis. (a,b) 9 WD. Human fetus of 45 mm GL. Horizontal section. Azan staining. (b) Enlargement of (a). A homogeneous interzone is observed in the incudopetrosal joint (IP) between the dorsal end of the short limb of the incus (SI) and the otic capsule (P). The tympanostapedial joint (IS) is in the interzone phase, and the cavity of incudomalleolar joint (IM) has become larger. For other abbreviations, see Figure 1

At 11 WD, the cartilaginous otic capsule also enlarged anteriorly on the head of the malleus and incus to form the tegmen tympani. The tegmen tympani closed the upper and posterior walls of the tympanic cavity, and thus formed the superior margin of the epitympanic recess. The tegmen tympani was inclined and with the squamous part of the temporal bone formed a wide petrosquamosal fissure (Figure 4c). The short limb of the incus was located in a recess or the future fossa incudis between the squamous part of the temporal bone and the otic capsule near the lateral semicircular canal and facial nerve (Figure 4c,d).

The otic capsule had an eminence or protrusion located the interzone with the short limb of the incus. The short limb of the incus was joined to the squamous part of the temporal bone by a condensed mesenchyme, which became the posterior ligament of the incus (Figure 4d).

At 12 WD, a trilaminar interzone was detected between the incus and otic capsule, such that the medial layer of the interzone appeared more lax than the eccentric layers (Figure 4e).

3.5 | Cavitation phase of the incudopetrosal joint

At 13 WD, a narrow cavity appeared in the incudopetrosal joint between the squamous part of the temporal bone and the lateral aspect of the short limb of the incus near the beginning of posterior ligament of incus. The ligaments of the incudomalleolar joint were established at this stage (Figure 5a).

At 14 and 15 WD, the intramembranous ossification of the squamous part of the temporal bone joined the petrous part to form the petrosquamosal fissure, resulting in complete closure of the lateral and posterior wall of the epitympanic recess. The short limb of the incus was connected to the squamous part by the anlage posterior ligament of the incus. The cavity of the incudopetrosal joint was wider than at 13 WD and the primordium of the capsule and synovial membrane appeared. A short ridge appeared to surround the articular surface of the otic capsule, beginning of the articular apophysis (Figure 5b).

3.6 | Change of the incudopetrosal joint to a syndesmosis

At 16 and 17 WD, endochondral ossification had begun in the petrous part of the temporal bone near the facial nerve but had not reached the short limb of incus. The short limb was located in the fossa incudis in the posterior wall of the epitympanic recess. The cavity of the incudopetrosal joint was reduced in size (Figure 6a,b). The end of the short limb of the incus was articulated with the petrous part medially and joined the squamous part of the temporal bone laterally by the future posterior ligament of the incus. The intraarticular and capsular ligament started to develop by this stage

(Figure 6c). The progress of the incudopetrosal syndesmosis was similar to that of the tympanostapedial joint.

At 25 WD, the incus joint surface appeared to be covered with hyaline cartilage-like tissue (remnant of the cartilage without ossification). The other articular surface was located on a small apophysis in the petrous part of the temporal bone, lateral to the fossa incudis, which was also covered with hyaline cartilage-like tissue. An intrinsic interosseous ligament joined both articular surfaces, and an extrinsic ligament, the posterior ligament of the incus, joined the lateral side of the end of the short limb of the incus to the squamous part of the temporal bone (Figure 7a-c).

In a near-term (34 WD) fetus, the incudopetrosal joint was located in the fossa incudis, in the angle or recess that formed the squamous part and the petrous parts of the temporal bone, in the posterior wall of the tympanic cavity, at a level superior to the tympanic membrane, or epitympanic recess. The joint was located lateral to the protrusion of the facial canal, which was closed by an intramembranous ossification plate, inferior and medial to the protrusion of the lateral semicircular canal (Figure 8a-c).

Figure 9 represents the phases of the development of the incudopetrosal joint. The representative results and variations of each stage are shown in Table 1.

4 | DISCUSSION

Although morphologically transient, we demonstrated a real joint with a cavity between the short limb of the incus and the otic

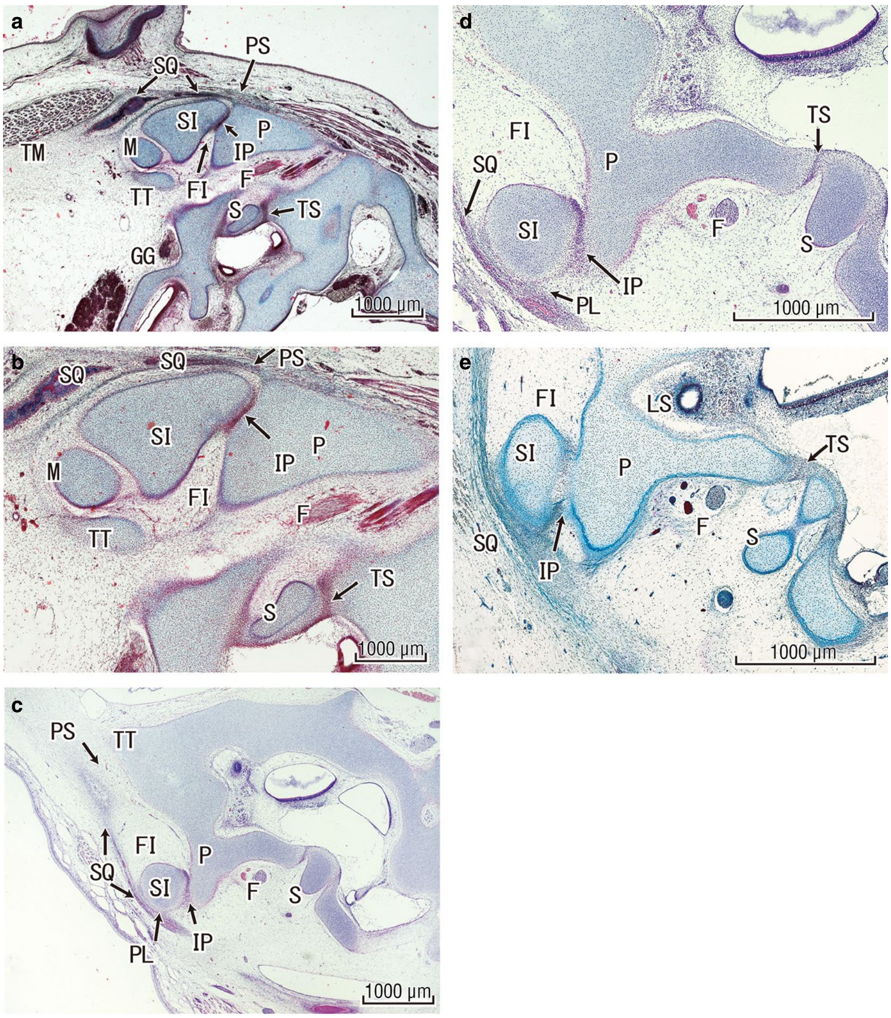

FIGURE 4 Establishment of the petrosquamosal fissure and closure of the posterior walls of the tympanic cavity. (a,b) 10 WD. Human fetus of 52 mm GL. Horizontal section. Azan staining. (b) Enlargement of (a). The petrosquamosal fissure (PS) has formed between the squamous part (SQ) and the petrous part (P, otic capsule) of the temporal bone. (c,d) 11 WD. Human fetus of 64 mm GL. Frontal sections. HE staining. (d) Enlargement of (c). The tegmen tympani (TT) has closed the upper and posterior walls of the tympanic cavity. The tegmen tympani (TT) has formed a wide petrosquamosal fissure (PS) with the squamous part of the temporal bone (SQ). The short limb of the incus (SI) is located in a recess, i.e. the future fossa incudis (FI). The otic capsule (P) has an eminence or protrusion where the interzone of the incudopetrosal joint (IP) is located. The posterior ligament of the incus (PL) has begun to develop as a dense mesenchyme between the squamous part of the temporal bone (SQ) and short limb of the incus (SI). (e) 12 WD. Human fetus of 74.5 mm GL. Frontal section. Azan staining. The incudopetrosal joint (IP) located in the fossa incudis (FI) is in the trilaminar interzone phase. For other abbreviations, see Figure 1

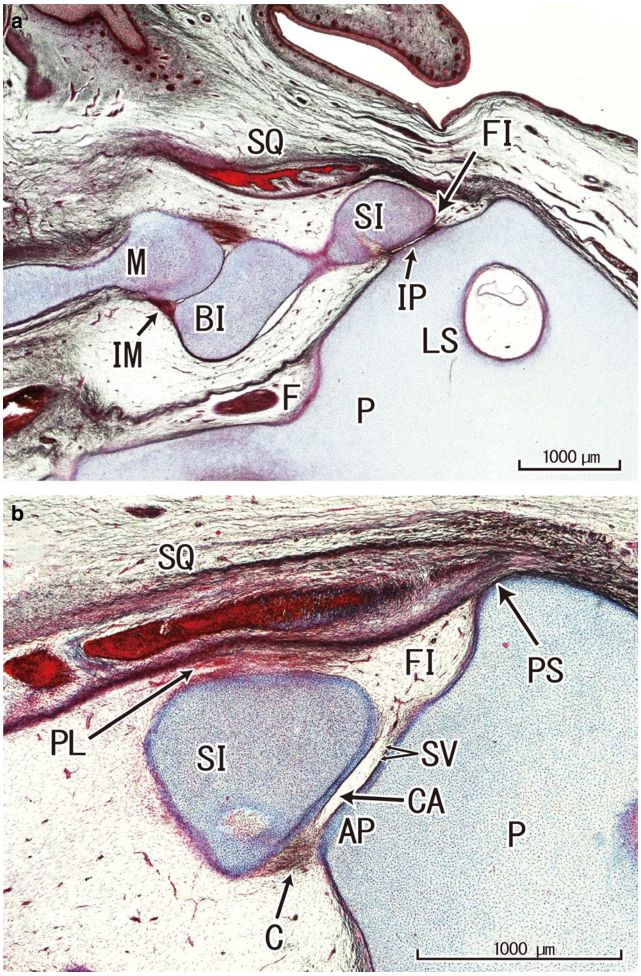

FIGURE 5 Cavitation phase of the incudopetrosal joint. (a) 13 WD. Human fetus of 105 mm GL. Horizontal section. Azan staining. The topographical anatomy of the tympanic cavity is observed at the level of the fossa incudis (FI), as well as the location and relationships of the incudopetrosal joint (IP) in the initial cavitation phase. (b) 14 WD. Human fetus of 120 mm GL. Horizontal section. Azan staining. The cavity (CA) of the incudopetrosal joint (IP) is wider and delimited by the initial capsule (C) and synovial membrane has appeared (SV). The anlage of the posterior ligament of the incus (PL) is visible between the short limb of the incus (SI) and the squamous part of the temporal bone (SQ). For other abbreviations, see Figure 1

capsule, i.e. the incudopetrosal joint. This appears to be the first report of a joint between the neurocranium and the first pharyngeal arch derivative (i.e. incus) in mammals. In contrast to the first pharyngeal arch, Reichert's cartilage does not form a joint with the otic capsule, but rather a fusion or connection (Rodríguez-Vázquez, 2005, 2009). Instead, the stapes, derived from the second pharyngeal arch, connects with the oval window, which is part of the neurocranium. Thus, the tympanostapedial joint or syndesmosis connects the second arch to the neurocranium, and not the first arch to the neurocranium. It is widely accepted that the primary jaw is composed of the quadrate and articular cartilages, and that it corresponds to the incudomalleolar joint in mammals (Table 2; Gaupp, 1913). The primary jaw contains three components: the

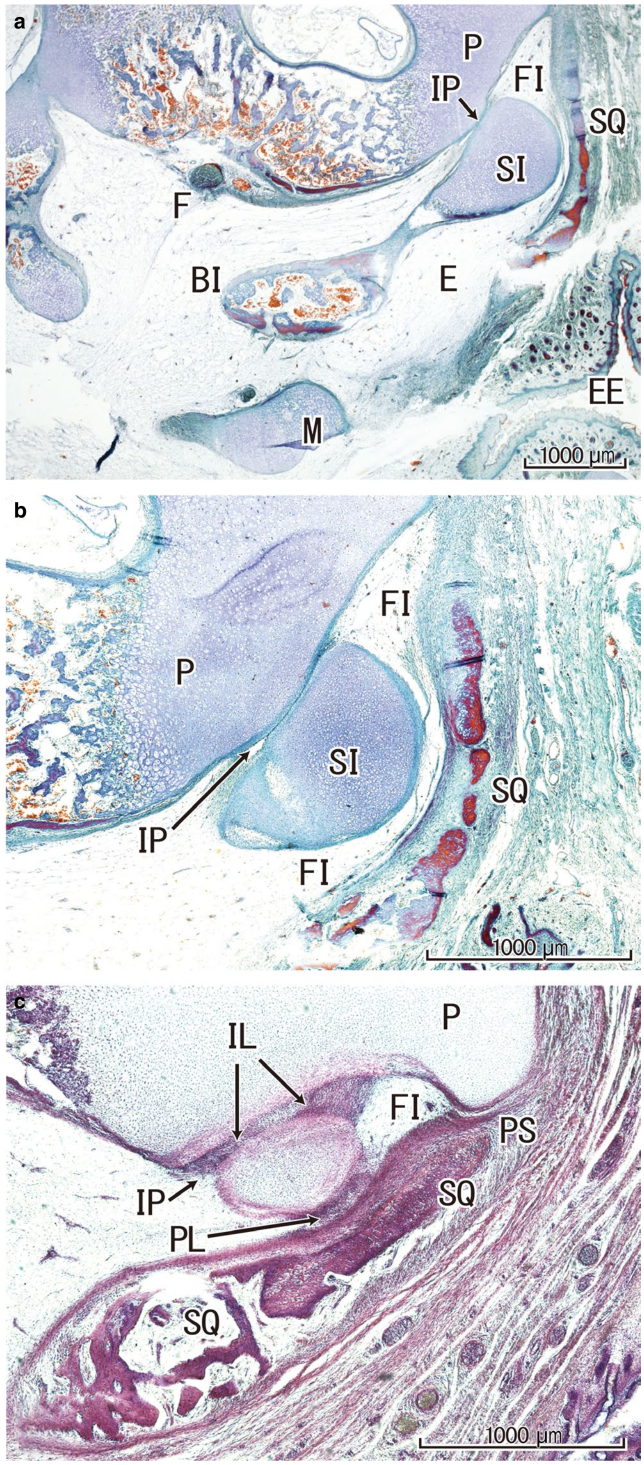

FIGURE 6 Change of the incudopetrosal joint to a syndesmosis. (a, b) 16 WD. Human fetus of 144 mm GL. Horizontal section. OF staining. (b) Enlargement of (a). Endochondral ossification has begun in the petrous part of the temporal bone (P) near the facial nerve (F) but has not reached the short limb of the incus (SI). The cavity of the incudopetrosal joint (IP) is reduced in size and located in the fossa incudis (FI) in the posterior wall of the epitympanic recess (e). (c) 17 WD. Human fetus of 150 mm. Horizontal section. HE staining. Development of the intraarticular and capsular ligament (IL) has begun. For other abbreviations, see Figure 1

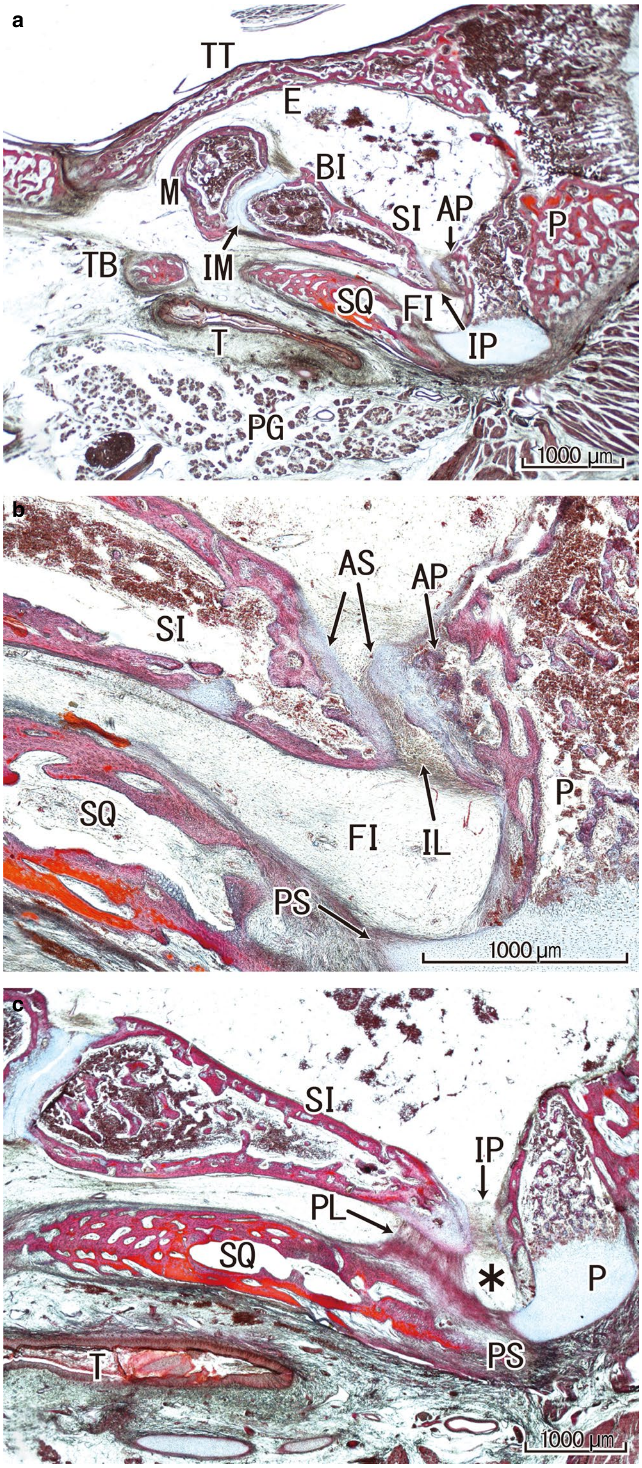

FIGURE 7 Incudopetrosal joint in a 25 WD human fetus. (a-c) Human fetus of 240 mm GL. Sagittal sections. Azan staining. (a) The most lateral section. (c) The most medial section. (b) Enlargement of (a). Topographical anatomy of the tympanic cavity. The incudomalleolar (IM) and incudopetrosal (IP) joints can be observed. The incudopetrosal joint (IP) has changed to a syndesmosis, an intrinsic interosseous ligament (IL) joins both articular surfaces, and an extrinsic ligament, the posterior ligament (PL) of the incus, has joined the lateral side of the end of the short limb of the incus (SI) to the squamous part of the temporal bone (SQ). * Posterior tympanic isthmus. For other abbreviations, see Figure 1

lower jaw, the upper jaw and another cartilage connecting to the neurocranium (reviewed by Takechi and Kuratani, 2010). In mammals, the former two are originate in the first arch and the last component in the second arch.

A basic dorso-ventral connection of pharyngeal arch derivatives is seen in sharks, involving a combination of the suprapharyngobranchial, epibranchial and ceratobranchial cartilages (Portmann, 1969) (Table 2). However, even in shark embryos, the jaw is formed through a combination of the first and second arches (Takechi and Kuratani, 2010). In mice, however, the outer edge of the stapedial footplate has a mesodermal origin (Thompson *et al.*, 2012). If the primary jaw model is based simply on a single origin or cell lineage, such as that involving the posterior arches in sharks, then a combination of Meckel's cartilage-malleus-incus (a new theory shown in Table 2 and Figure 10) might be better than the classical combination, i.e. the malleus-incus-stapes. Meckel's cartilage-malleus complex is a transient but definite histological structure in human embryos (Rodríguez-Vázquez *et al.*, 1992; Ozeki-Satoh *et al.*, 2016). Therefore, does a transient joint-like structure exist between Meckel's cartilage and the malleus?

Meckel's cartilage regresses and undergoes transformation into the sphenomandibular ligament as well as the anterior ligament of the malleus, and these ligaments are separated from the malleus by the tympanosquamosal fissure (Rodríguez-Vazquez et al. 1992). However, this fissure does not represent a simple gap between Meckel's cartilage and the malleus. The tegmen tympani (part of the neurocranium; the antero-inferior end of the petrosal portion of the temporal bone) descends inferiorly and wedges into the fissure to close it. Moreover, the discomalleolar ligament from the definitive or final jaw is the most likely to provide inferior traction for the tegmen tympani (Rodríguez-Vázquez *et al.*, 2011). Therefore, in humans, development and growth of the definitive jaw seems to require mechanical cooperation with separation between Meckel's cartilage and the malleus (Figure 10). The tegmen tympani finally closes the middle ear without any possibility of articulation between these two structures.

In fossils of early Cretaceous mammals, Wang *et al.* (2001) studied the transition from Meckel's cartilage-malleus complex to the definitive jaw and identified an ossified Meckel's cartilage that was still connected to the middle ear. Those authors suggested that the function of the cartilage could be to move the mandible. Therefore, any movements at this stage of development must involve this joint, which would be the primary jaw articulation. As stated by Kuhn (1971) and Maier (1987), in marsupial neonates the elasticity of the proximal portion of Meckel's cartilage most likely plays a mechanical role in the small movements and stresses involved in the act of suckling. Unfortunately, there was no evidence of the incudopetrosal joint in these animals.

Prior to initial disconnection, the incus and otic capsule were continuous or fused at the future posterior wall near the lateral semicircular canal and lateral to the facial nerve. Such a continuity of tissues is commonly seen in the fetal development of synovial joints (Hamilton and Mossman 1975, O'Rahilly and Müller, 1996). Likewise, the process of cavitation of the incudopetrosal joint was similar to

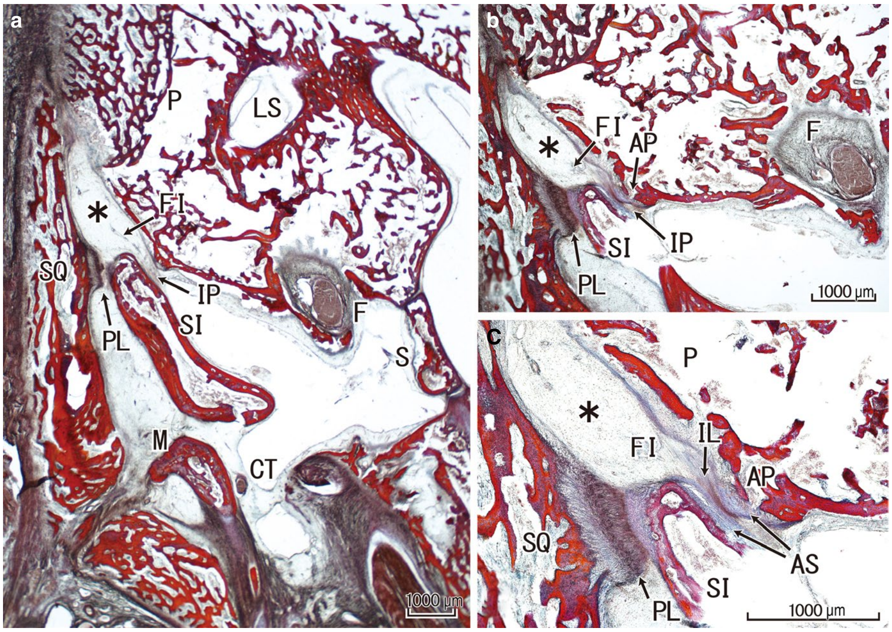

FIGURE 8 Incudopetrosal joint in a 34 WD human fetus. (a-c) Human fetus of 340 mm. Horizontal sections. Azan staining. (a) The most superior section. (c) The most inferior section. (c) Enlargement of (b). Topographical anatomy of the incudopetrosal joint (IP) at the tympanic cavity. The incudopetrosal joint (IP) is located in the fossa incudis (FI) in the angle or recess that forms the squamous part (SQ) and the petrous parts (P) of the temporal bone, in the posterior wall of the tympanic cavity at the epitympanic recess lateral to the protrusion of the facial canal and facial nerve (F), and inferior and medial to the lateral semicircular canal (LS). * Posterior tympanic isthmus. For other abbreviations, see Figure 1

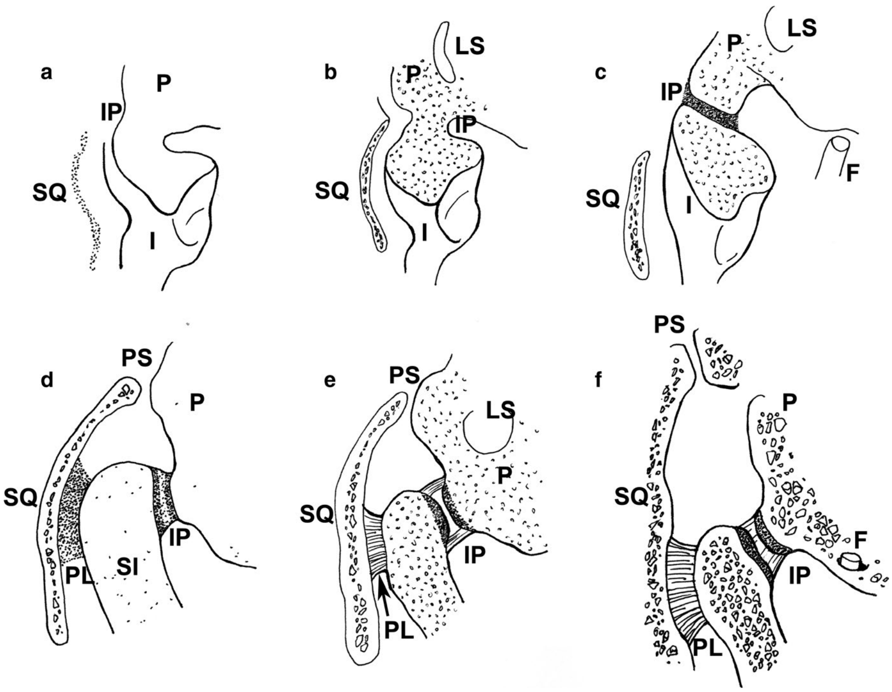

FIGURE 9 Diagrammatic representation of the steps of development of the incudopetrosal joint (IP). (a) Precartilaginous union incus-otic capsule (7WD). (b) Cartilaginous union between the incus and the otic capsule (8 WD). (c) Homogeneous interzone phase (9–11 WD). (d) Trilaminar interzone phase (12 WD). (e) Cavitation phase (13–15 WD). (f) Joint syndesmosis phase (17–34 WD). For other abbreviations, see Figure 1

that of other synovial joints, occurring via an interzone phase followed by a trilaminar configuration (O'Rahilly and Müller, 1996) (Figure 9). Mitrovic (1978) pointed out that intrinsic factors are important for differentiation in the primitive stages of joint cavitation, and joint movements seem to be necessary for differentiation and maintenance of the joint cavity. Is functional demand for vibration and movement of the fetal ear ossicles necessary for development of the incudopetrosal joint?

Articulations of ear ossicles are characterized by elastic fiber-rich ligaments that are resistant to vibration (Kawase *et al.*, 2012). Since the elastic fibers appear early in joint development (Takanashi *et al.*, 2013), ear ossicles are most likely to vibrate and even move at the joints *in utero*. As the development of the incudopetrosal joint advanced at the same time or slightly later than the other ear ossicle joints, joint development may be coordinated with ossicle vibration. However, in contrast to the other joints comprising a combination of concave and convex surfaces, the incudopetrosal joint bears flat articular surfaces containing the intraarticular ligament. Moreover, degeneration of the joint cavity began at midterm and had disappeared by term. Therefore, development of the incudopetrosal joint seemed to require little or no functional demand for conduction of vibration.

TABLE 2 Three components of the primary jaw in relation to the shark and mammal

	Basic hypothesis	Shark	Mammal	New theory
To neurocran	Hyomandibular	Suprapharyngobranchial	Stapes	Incus
Upper jaw	Quadrate	Epibranchial	Incus	Malleus
Lower jaw	Articular	Ceratobranchial	Malleus	Meckel´s cartilage

Note: 'To neurocran' indicates a bony or cartilage component articulating with the neurocranium.
The basic evolutionary hypothesis is from Gaupp (1913) and Goodrich (1930).
The shark branchial skeleton is based on Portmann (1969).

The growing short limb of the incus appeared to accelerate expansion of the epitympanic recess of the tympanic cavity. The morphology of the posterior wall of the tympanic cavity seemed to be determined by the disposition of the short limb of the incus, behind the tip of which, the posterior tympanic isthmus is located (Palva and Johnsson, 1995; Palva and Ramsay, 1996). We found a boundary of the posterior tympanic isthmus, which passed through a narrow space between the transient incudopetrosal joint and the developing posterior ligament of the incus (asterisks in Figures 7c and 8a,c). Palva and Ramsay (1996) showed that the isthmus was small and enclosed by a thin membrane in adults. The opening of the posterior isthmus provides an auxiliary route for aeration of the epitympanum and mastoid through the incudal fossa (Palva and Ramsay, 1996).

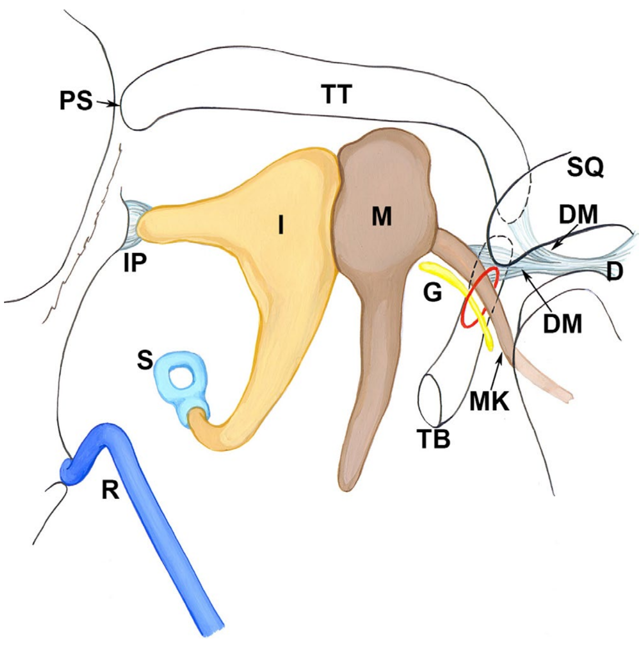

FIGURE 10 Lateral view diagrammatic representation of the incudopetrosal joint and the ossicles of the middle ear in midterm human fetuses. The incus (I) connects with the neurocranium (P, petrous part of the temporal bone) via the incudopetrosal joint (IP). The derivatives of the first arc (I, incus) are colored light brown, and the cartilage of the first pharyngeal arch (MK, Meckel´s cartilage) and its derivative malleus (M) dark brown. The derivative of the second pharyngeal arch (S, stapes) is colored light blue, and the cartilage of the second arch (R, Reichert's cartilage) dark blue. The Meckel cartilage (MK) passes through the tympanosquamosal fissure (red circle). The discomalleolar ligament (DM) connects the disk of the temporomandibular joint (D) to the inferior process of the tegmen tympani (TT) and descends into the fissure to close it (Rodríguez-Vázquez *et al.*, 2011). For other abbreviations, see Figure 1

Observations of five late-stage fetuses demonstrated that the incudopetrosal joint changed to a syndesmosis. The joint surfaces appeared to be covered with hyaline cartilage-like tissue. The petrous articular surface was located in a small apophysis. An intrinsic interosseous ligament joined both articular surfaces, and an extrinsic ligament, the posterior ligament of the incus, joined the lateral side of the end of the short limb of the incus to the squamous part of the temporal bone. This is the first anatomical description of this joint.

According to Proctor (1964) and Aimi (1971, 1978), the posterior ligament of the incus can be represented as parallel slender bands arising from the tip of the short limb of the incus. However, Palva and Ramsay (1996) reported that the ligament is composed of broad and strong bands directed along a mesiolateral axis, bridging the posterior portion of the short limb and the medial and lateral wall of the epitympanic recess. In the present study, the posterior ligament extended in an almost frontal plane and corresponded to the union between the short limb and the lateral wall of the tympanic cavity on the squamous part of the temporal bone. Palva and Ramsay (1996) noted that the medial portion of the ligament actually corresponded to the interosseous ligament within the incudopetrosal joint, in agreement with our observations.

ACKNOWLEDGEMENTS

The authors are grateful to Cristina Navarro Collin for making the drawings.

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DATA AVAILABILITY STATEMENT

The data have been shared.

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How to cite this article: Rodríguez-Vázquez JF, Yamamoto M, Kim JH, Jin Z-W, Katori Y, Murakami G. The incudopetrosal joint of the human middle ear: a transient morphology in fetuses. *J. Anat*. 2020;237:176–187. https://doi.org/10.1111/joa.13181