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Development of the Human Incus With Special Reference to the Detachment From the Chondrocranium to be Transferred into the Middle Ear

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ABSTRACT

The mammalian middle ear represents one of the most fundamental features defining this class of vertebrates. However, the origin and the developmental process of the incus in the human remains controversial. The present study seeks to demonstrate all the steps of development and integration of the incus within the middle ear. We examined histological sections of 55 human embryos and fetuses at 6 to 13 weeks of development. At 6 weeks of development (16 Carnegie Stage), the incus anlage was found at the cranial end of the first pharyngeal arch. At this stage, each of the three anlagen of the ossicles in the middle ear were independent in different locations. At Carnegie Stage 17 a homogeneous interzone clearly defined the incus and malleus anlagen. The cranial end of the incus was located very close to the otic capsule. At 7 and 8 weeks was characterized by the short limb of the incus connecting with the otic capsule. At 9 weeks was characterized by an initial disconnection of the incus from the otic capsule. At 13 weeks, a cavity appeared between the otic capsule and incus. Our results provide significant evidence that the human incus developed from the first pharyngeal arch but independently from Meckel's cartilage. Also, during development, the incus was connected with the otic capsule, and then it was detached definitively. The development of the incus in humans provides evidence that this ossicle is homologous to the quadrate. Anat Rec, 301:1405–1415, 2018. © 2018 Wiley Periodicals, Inc.

Abbreviations: AV = anterior cardinal vein (primary head sinus); BI = body of the incus; CP = crista parotica of the otic capsule; CT = chorda tympani; ER = epitympanic recess; F = facial nerve; FG = first pharyngeal groove; FI = incudo-capsular fissure; FP = first pharyngeal pouch; G = goniale; GG = geniculate ganglion; GP = greater petrosal nerve; HA = hyoid artery; I = incus; IC = internal carotid artery; IH = interhyale; IM = incudomalleolar joint; IS = incudostapedial joint; LI = long limb of the incus; LP = lenticular process of the incus; M = malleus; MA = mandibular artery; ME = meatus acusticus externus; MK = Meckel's cartilage; OC = otic capsule; O = otic vesicle; PH = pharynx; R = Reichert's cartilage; S = stapes; SA = stapedial artery; SI = short limb of the incus; SM = stapedius muscle; SO = supraorbital artery; TG = trigeminal ganglion; TT = tensor tympani muscle; Vb = maxillary nerve; Vc = mandibular nerve; IX = glossopharyngeal nerve; X = vagus nerve

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The mammalian middle ear constitutes one of the most fundamental features that define this class of vertebrates. The origin of the mammalian ear ossicles is a classic example of gradual evolutionary change and has received considerable attention (Allin, 1975; Maier, 1989; Allin and Hopson, 1992; Rowe, 1996; Takechi and Kuratani, 2010). During their development, embryos of many species repeat evolutionary stages of their ancestors (Haeckel, 1866). From the perspective of comparative morphology, the vertebrate head exhibits a series the pharyngeal arches. The first pharyngeal arch, the mandibular arch, comprises the upper and lower jaws, called the palatoquadrate and Meckel's cartilage, respectively (Goodrich, 1930). Reichert (1837) formulated a hypothesis that persists today, that is, that the mammalian ossicles, the malleus and incus, were derived from cartilages equivalent to the lower and upper jaw elements in other amniotes: the articular and quadrate. Gaupp (1913) pointed out that, unlike the nonmammalian jaw in which the "primary jaw" consists of the quadrate and articular, mammals have a unique "secondary jaw" between two dermal elements, that is, squamosal and dentary (Gaupp, 1911, 1913). Gaupp's morphological insight influenced subsequent evolutionary studies on the middle ear, based on the fossil record and embryology. An excellent historical review and explication of this subject has recently been published (Maier and Ruf, 2016).

Fossil records and developmental events in marsupials have provided further evidence to support the primary jaw-joint origin of the mammalian middle ear described in the Reichert–Gaupp theory. In basal mammaliaformes, postdentary bones have diminished significantly but are still attached to the dentary, serving a dual function for hearing and feeding (Allin, 1975). Furthermore, it is reported that the offspring of marsupials do not possess a temporomandibular joint and retain an operational primary jaw joint (Crompton and Parker, 1978). However, there is no evidence of homology between the incus in humans and the nonmammalian quadrate.

The numerous and often controversial publications regarding the differentiation of organogenesis of human-ear ossicles were carefully reviewed and summarized by Broman (1899), Gaupp (1899), Van der Klaauw (1924), and Maier and Ruf (2016). Most authors agree that the incus and malleus are derived from the first pharyngeal arch (Gaupp, 1899). However, the slightly different opinion of Broman (1899) proposes that both the incus and malleus derive from the first pharyngeal arch, but the location of the incus anlage differs from that of the malleus anlage (Fig. 1). Van der Klaauw (1924) claimed that the information on the origin of the incus was poor since it was difficult to define the shape of the incus anlage in the blastematic stage.

Although recent reports also provide a better understanding of the origins of the human-ear ossicles (Rodríguez-Vázquez et al., 1991, 2011; Rodríguez Vázquez, 2005, 2009), the origin of the incus in the human remains controversial: (1) an single-origin theory in which the first pharyngeal arch or Meckel's cartilage develops into both of the incus and malleus (Reichert, 1837; Hamilton and Mossman, 1975; Corliss, 1979; Moore and Persaud, 1999; Larsen, 2003; Sadler, 2004; Burford and Mason, 2016); and (2) a double-origin theory in which head and neck of the malleus as well as the body of incus arise from the first pharyngeal arch, while the second arch gives rise to the handle of the malleus and long process of the incus (Anson et al., 1960; Hanson et al., 1962; Hough, 1963; Cousins and Milton, 1988; Nomura et al., 1988; Ars, 1989; Louryan, 1993; Takeda et al., 1996; Whyte et al., 2009).

The present study describes all the developmental steps of the human incus in order to provide a better understanding of (1) the origin of the incus and (2) how the incus was involved or transformed from an element of the primary jaw structure to one of the middle-ear ossicles. The present results support the reconsideration of the evolution of the middle ear and jaw.

MATERIALS AND METHODS

The study complies with the provisions of the Helsinki Declaration (World Medical Association, 2013). A total of 55 embryos and foetuses from the collection of the Embryology Institute of the University Complutense of Madrid (Spain) were studied. In the embryos, the greatest length (GL) ranged from 10 to 31 mm (Carnegie Stages (CS) 16 to 23; 6 to 8 weeks of development (WD). In the foetuses, the GL ranged from 33 to 100 mm (9–13 WD). The parameters used to determine the postconceptional age were GL and external and internal criteria (O'Rahilly and Müller, 2010). 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 from 7 to 25 μm thick, depending on specimen size. The sections were stained with hematoxylin and eosin, azan and Bielschowsky.

RESULTS

Initial Appearance and Topographical Delimitation of the Incus Anlage 6WD (CS 16)

The first and second pharyngeal arches were delimited by the cranial part of the cardinal anterior vein (primary head sinus). The incus anlage appeared as a low-density mesenchymal condensation, which was located at the cranial end of the first pharyngeal arch. The primary head sinus, facial nerve, and stapes anlage were located medial to the incus anlage (Fig. 2A,AA and

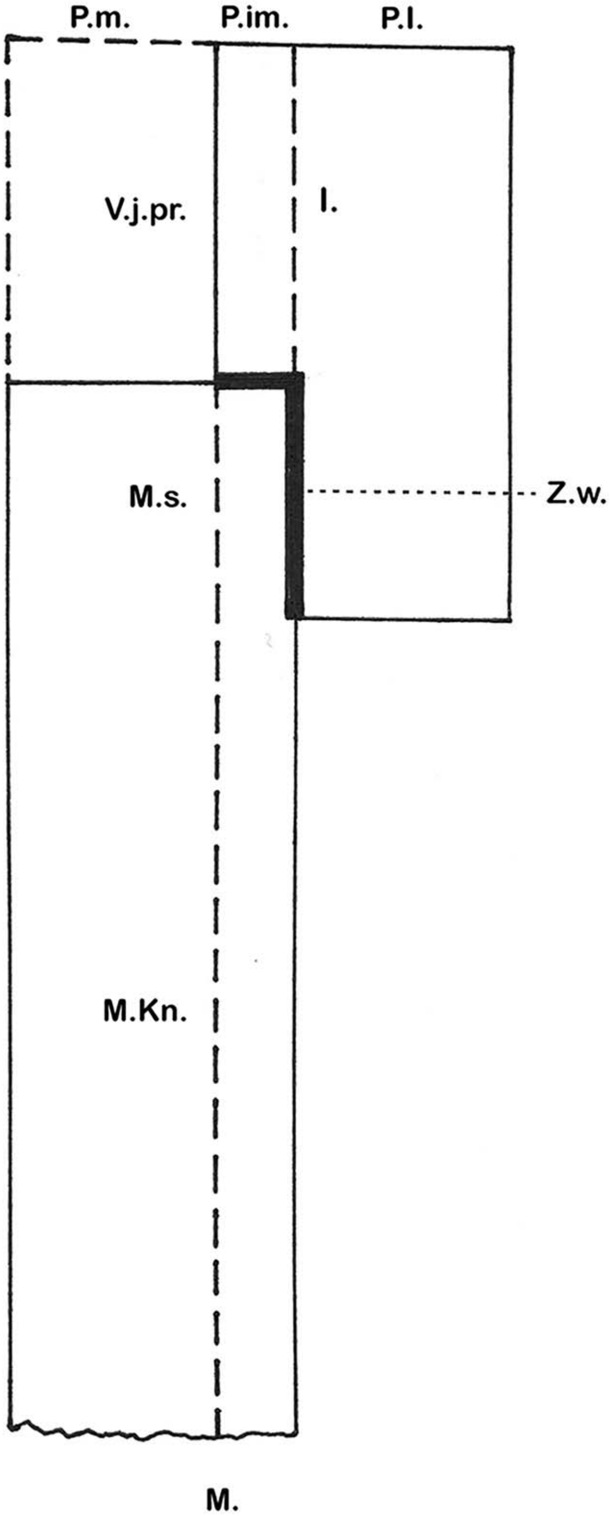

Fig. 1. Drawing of parts of the first arch according to Ivar Broman, 1899. M, mandibular arch; P.m., Pars medialis; P.l., Pars lateralis; P.im., Pars intermedia; V.j.pr., primitive jugular vein; I., anlage of the incus; M.s., anlage of the malleus; M.Kn., Meckel's cartilage; Z.w., Zwischenscheibe.

drawing). The stapedial artery crossed the stapedial anlage and passed the caudal to the facial nerve and primary sinus head. Caudally, the incus anlage was located at the level of the bifurcation of supraorbital and mandibular arteries, which arose from the stapedial artery. These arteries penetrated the mandibular arch (Fig. 2B and drawing). The supraorbital artery ascended into the first pharyngeal arch medially to the incus anlage (Fig. 2AA,B and drawing).

The cranial end of the mesenchymal condensation, which continuous with the Meckel's cartilage (the malleus anlage), was located inferior to the incus anlage, at the level of the future meatus acusticus externus (Fig. 2D and drawing). Undifferentiated mesenchyme was located between the incus and malleus anlagen. The mandibular branch of the artery passed through this mesenchyme (Fig. 2C and drawing). The incus anlage was separated from the malleus anlage in the first pharyngeal arch. At this stage, each three anlagen of the ossicles of the middle ear were independent in different locations (Fig. 2).

Delimitation of the Parts of Incus Anlage and Connection With Otic Capsule of the Chondrocranium 6 WD (CS 17–18)

At the CS 17, the connection was established between the incus and the malleus, which were separated by an undifferentiated mesenchyme in the previous stage. This connection was delimited by homogenous interzones. The cranial end of the incus anlage consisted of mesenchymal condensation. It was located close to the otic capsule and lateral to course of the facial nerve and the cardinal anterior vein (primary head sinus) (Figs. 3A and 9A). Caudally, the initial homogeneous interzones, the future incudomalleolar (incudostapedial) joint, separated the incus anlage from the malleus (stapes) anlage (Figs. 3B,BB and 9A). The anlage of the malleus was continuous with Meckel's cartilage without any demarcation (Figs. 3C and 9A). This area could be inferred from the nearby course of the chorda tympani (Fig. 3C). The interhyale was visible as a mesenchymal condensation located in front of the facial nerve. This structure established continuity between the stapedial anlage and the blastematic condensation giving rise to Reichert's cartilage (Figs. 3B,BB and 9).

At CS 18, the short limb of the incus, which was a dorsal extension of the body, joined the otic capsule by mesenchymal condensation (Fig. 4A). This mesenchymal condensation was located on the lateral part of the otic capsule (Fig.4A). The anlage of the stapedial limb was separated by the stapedial artery originating from the hyoid artery medially to the facial nerve (Fig. 4A). The long limb of the incus (Fig. 4B) was located caudally along the course of the facial nerve and hyoid artery, which gave rise to the stapedial artery (Fig. 4B). The thickened caudal end of the long limb corresponds to the lenticular process (Fig. 4B). A homogeneous interzone clearly delimited the incus anlage and the anlage of the malleus (cranial end of Meckel's cartilage) (Fig. 4B).

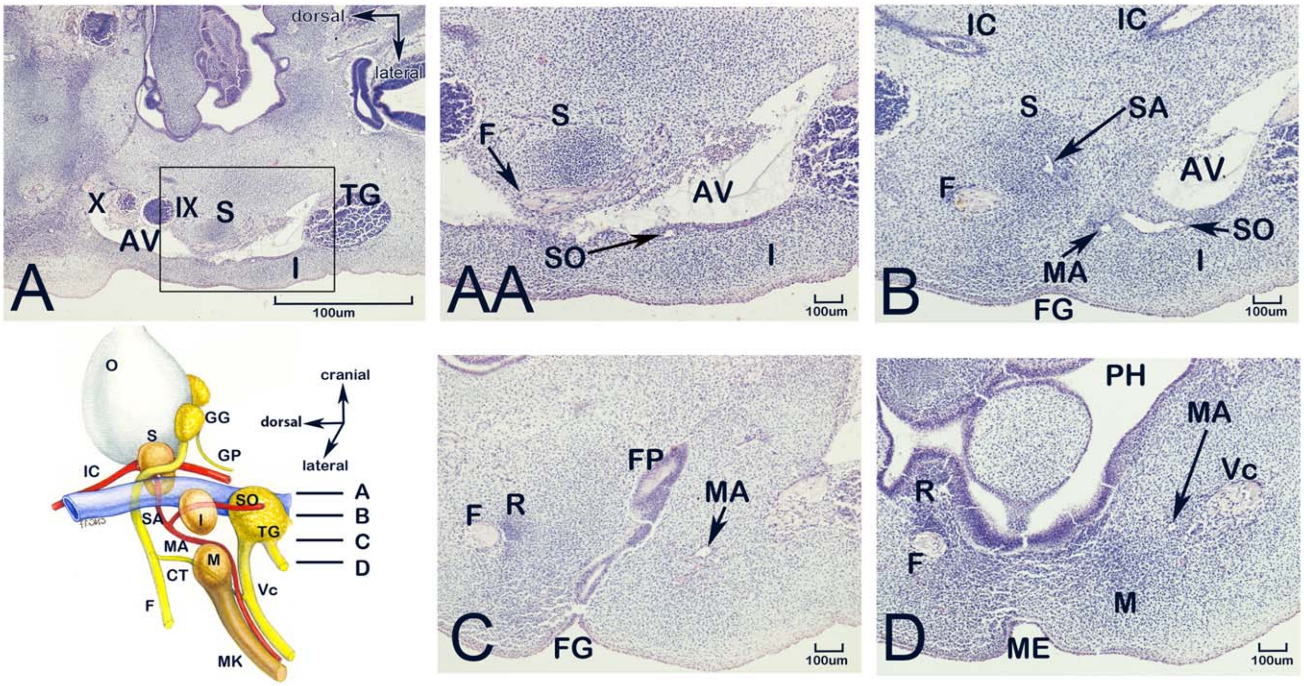

Fig. 2. Initial appearance and topographical delimitation of the incus anlage. 6 WD. In the diagrammatic representation of the topographical relationship of the anlages of the incus (I), malleus (M), and stapes (S), the levels of section A, B, C, and D have been marked corresponding to the same figures. Human embryo of 11 mm GL (16 CS). Horizontal sections. Panel (AA) is high magnification of the square in Panel (A). Panels (AA–D) are same magnifications. Panels (A,AA) is the most cranial level and panel (D) is the most caudal. The anlage of incus (I) was located at the cranial end of the first pharyngeal arch (Panels A,AA). The anterior cardinal vein (primary head sinus) (AV) delimited the cranial ends of the first and second pharyngeal arches. In the second pharyngeal arch was located the stapedial anlage (S) and facial nerve (F) (Panels A,AA). Caudally, the incus anlage was located at the level of the bifurcation of stapedial artery (SA) in supraorbital (SO) and mandibular (MA) branches (Panel B). The malleus anlage (M) was located inferior to the incus anlage (I), at the level of the future meatus acusticus externus (ME), dorsally to the mandibular nerve (Vc) and mandibular branch (MA) of the stapedial artery (Panel D). The mandibular branch (MA) of the stapedial artery passed through of the undifferentiated mesenchyme which was located between the incus and malleus anlagen (Panel C). CT, chorda tympani; FG, first pharyngeal groove; FP, first pharyngeal pouch; GG, geniculate ganglion; GP, greater petrosal nerve; IC, internal carotid artery; MK, Meckel's cartilage; O, otic vesicle; PH, pharynx; R, Reichert's cartilage; TG, trigeminal ganglion; IX, glossopharyngeal nerve; X, vagus nerve.

Precartilaginous and Cartilaginous Phase of the Connection of the Incus to the Otic Capsule 7–8 WD

At 7 WD (CS 20), the crista parotica, a lateral protrusion in the otic capsule, first appeared. The precartilaginous connection was distinguishable between the short limb of the incus and crista parotica of the otic capsule (Fig. 5A–C). This connection formed an S-shape and was identified in several embryos from the same stage. This connection was located lateral to the horizontal course of the facial nerve and the stapedial artery, which had begun a process of involution during intrastapedial course (Fig. 5A,AA). An "incudo-capsular fissure" was evident between the area where the short limb of the incus joined the otic capsule and the body of the incus (Fig. 5AA,B). The future incudomalleolar joint was identified as a trilaminar interzone between the body of the incus and the head of the malleus (Fig. 5C).

At the initial stage of 8 WD (CS 22), the ossicular anlages were formed. Cartilaginous connection was observed between the incus and otic capsule (Figs. 6A,AA and 9B). The "incudo-capsular fissure" narrowed, due to the growth of both the incus and otic capsule (Fig. 6AA). In the interzone of the incudomalleolar joint, between the body of the incus and the head of the malleus, a small cavity was identified (Fig. 6AA). At 8 WD (CS 23), the cavity of incudomalleolar joint became larger and was also found in its caudal part (Fig. 6B). The continuity between the malleus and Meckel's cartilage was clearly evident and the os goniale was located caudally to Meckel's cartilage (Figs. 6B and 9B).

Disconnection Phase of the Incus to the Otic Capsule 9–13 WD

At 9 WD, we detected the initial disconnection of the incus by a "incudocapsular" homogeneous interzone (Figs. 7A,AA). This interzone was located between the dorsal end of the short limb of the incus and the otic capsule and lateral to the course of the facial nerve. At this point, the cavity of the incudomalleolar joint was larger than at 8 WD (Fig. 7A).

At 11 WD, the dorsal end of the short limb of incus was located dorsally to the facial canal in the future fossa incudis of the epitympanic recess (Fig. 7B).

At 12 WD, the delamination of the interzone was detected between the incus and otic capsule and a small articular cavity appeared (Fig. 8A).

At 13 WD, the disconnection between the incus and the otic capsule became larger than 12 WD and a small

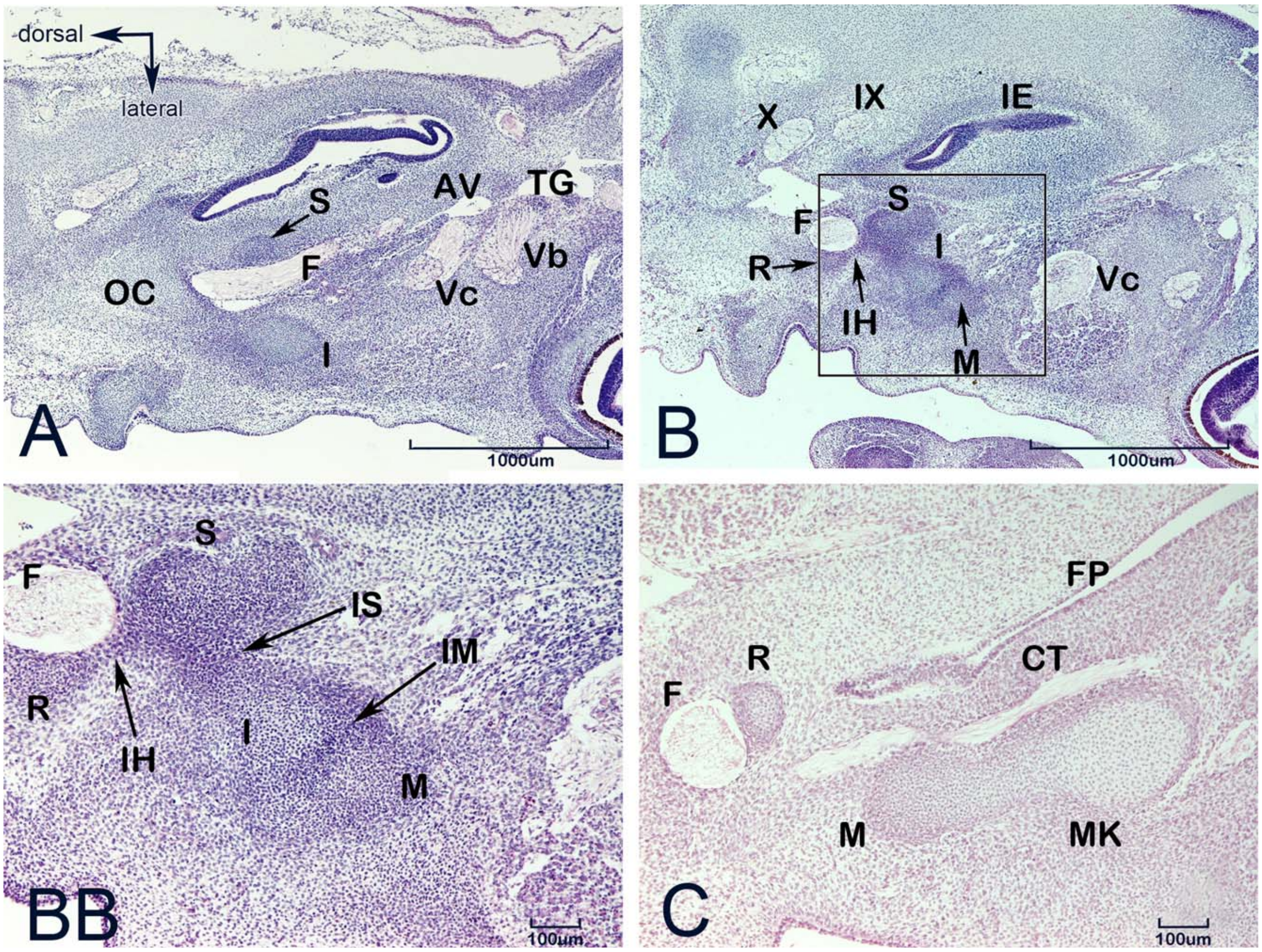

Fig. 3. The anlage of the incus was located very close to the otic capsule. 6 WD. Human embryo of 12.5 mm GL (17 CS). Horizontal sections. Panel (BB) is high magnification of the square in Panel (B). Panels (BB,C) are same magnifications. Panel (A) is the most cranial level and panel (C) is the most caudal. The anlage of incus (I) consists of a mesenchymal condensation clearly defined. Cranially it was located very close to the otic capsule (OC) and lateral to the course of the facial nerve (F) (Panel A). Caudally, the anlage of the incus (I) was delimited from the stapes (S) and the malleus anlagen (M) by the initial of the homogeneous interzones incudostapedial (IS) and incudomalleolar (IM) (Panels B,BB). Contrarily, the anlage of the malleus (M) was continuous with Meckel's cartilage (MK) close the course of the chorda tympani (CT) (Panel C). The interhyale (IH) joined the stapedial anlage with the Reichert's cartilage (R) (Panels B,BB). AV, anterior cardinal vein (primary head sinus); FP, first pharyngeal pouch; Vb, maxillary nerve; Vc, mandibular nerve; TG, trigeminal ganglion; IX, glossopharyngeal nerve; X, vagus nerve.

joint cavity appeared between the otic capsule and the short limb of the incus (Figs. 8B and 9C). The morphological characteristics of this joint corresponded to a syndesmosis. This area was located close to the facial nerve in the middle ear (Fig. 8B).

DISCUSSION

The transference of postdentary jaw elements to the cranium of mammals as auditory ossicles is one of the central topics in the evolutionary biology of vertebrates. Studies of mammal-like reptiles show that the anatomical patterns of the middle ear have gradually transformed elements from the primary jaw joint into mammalian middle-ear components (Gregory, 1913; Westoll, 1944, 1945; Hopson, 1966; Allin,1975; Takechi and Kuratani, 2010; Meng et al., 2011; Maier and Ruf, 2016). Studies in humans have pointed out that the incus originated from Meckel's cartilage either totally (Hamilton and Mossman, 1975; Corliss, 1979; Moore and Persaud, 1999; Larsen, 2003; Sadler, 2004; Burford and Mason, 2016) or partially (Nomura et al., 1988; Ars, 1989; Takeda et al., 1996; Whyte et al., 2009).

Our present study demonstrates first all the steps of development and integration of the incus into the middle ear, with special attention to its connective relationship with the otic capsule and detachment or disconnection from the otic capsule of the chondrocranium. For the initial steps, at 6 WD (CS 16), the cranial ends of the first and second pharyngeal arch were delimited by the primary head sinus. The incus anlage was found to be lateral to this vein at the cranial end of the first pharyngeal arch. The continuity between the incus and malleus anlagen was not identified. There was an undifferentiated mesenchyme between the incus and malleus anlagen, and the mandibular branch of the stapedial

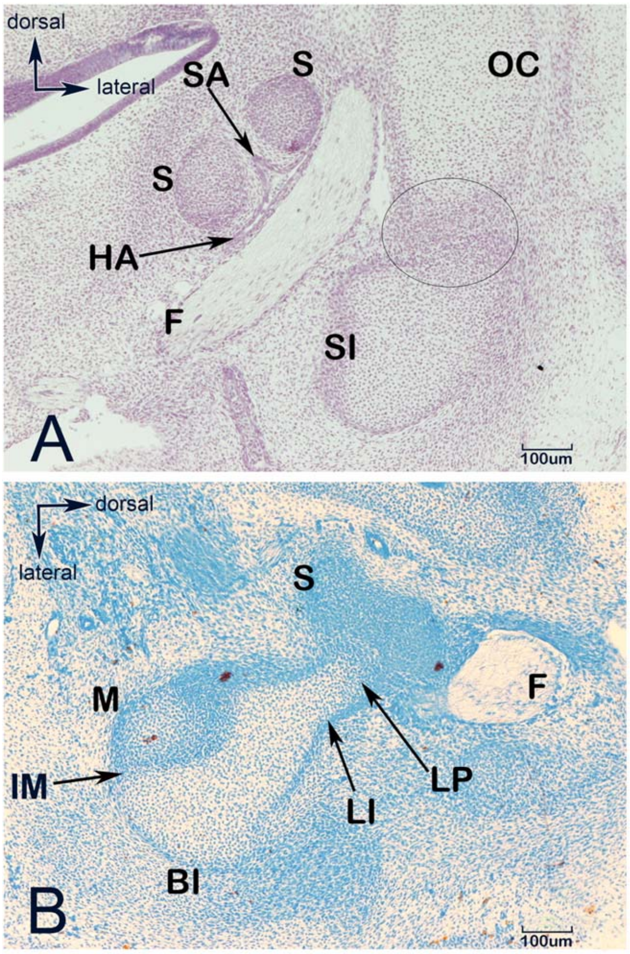

Fig. 4. Delimitation of the parts of the anlage of the incus and connection with otic capsule. 6 WD. Human embryo of 16 mm GL (18 CS). Horizontal sections. Panel (A) is the most cranial level and panel (B) is the most caudal. The short limb of the incus (SI) joins the otic capsule (OC) by condensed mesenchyme (surrounded by oval). The long limb of the incus (LI) (Panel B) was located caudally to the course of the facial nerve (F) and hyoid artery (HA), which gave rise to the stapedial artery (SA) (Panel A). The caudal end of the long limb of the incus was identified as the lenticular process (LP). The homogeneous interzone (IM) delimited the body of the incus (BI) and the malleus (M) (Panel B). S, stapes.

artery passed through this mesenchyme. Both the incus and malleus anlagen were located in the first pharyngeal arch. However, the location of the incus anlage was different from that of the malleus anlage, or the origin of the incus is independent from Meckel's cartilage. There was also no connection between the stapes and incus anlagen. Therefore, we agree with Broman (1899), who contended that the proximal portion of the lateral part (pars lateralis) of the blastema of the mandibular arch region gives rise to the anlage of the incus. The proximal end of the medial part (pars medialis) is for the course of the primitive jugular vein and the distal end of the medial part give rise to the anlage of the malleus and Meckel's cartilage (Fig. 1). Mesenchyme in the first pharyngeal arch seems to form the incus as well as Meckel's cartilage, but the origin of the first pharyngeal arch should not be considered equivalent to the origin of Meckel's cartilage. According Ozeki-Satoh et al. 2016, the incus was no contiguous with the malleus at CS 19.

The malleus and incus in mice develop from a single condensation, which immediately splits to form the two ossicles (Amin and Tucker, 2006). The ossicles (the malleus and incus) have different genetic identities and joint markers express between the two (Amin and Tucker, 2006). Genetically, the malleus and incus are, therefore, distinguishable at a stage when morphologically they appear as a single entity. In another paper (Amin et al., 2007), these authors mention that the malleus and incus are still continuous at embryonic day 13.5 of mice. In that study, at embryonic days 13.5–15.5, proliferation cells were observed in the malleus and incus by using PCNA staining, but the developing incudomalleal joint was characterised by a lack of proliferation (Amin et al., 2007). Although the lineage could not be determined from an analysis of histological sections, we consider it possible to determine the topographical areas of the first pharyngeal arch and their relationships.

At 6 WD (CS 17), a homogeneous interzone was clearly delimited between the incus and malleus anlagen. The cranial end of the incus was located very close to the otic capsule. Caudally, the malleus anlage was continuous with Meckel's cartilage without any demarcation (Fig. 9A). Broman (1899) schematically represented this interzone that denominated "Zwischenscheibe" (Fig. 1). The interzones becomes an important signaling center to the opposing elements, which can regulate growth through such factors as GDF-5 (Archer et al., 2003). The skeletal elements express type II collagen, while the joint interzone is negative type II collagen (Craig et al., 1987). Broman (1899) emphasized that the incus would have a precartilaginous nucleus that differed from the malleus and Meckel's cartilage.

At CS 18, we found a mesenchymal connection between the chondrocranium (i.e., the otic capsule) and the short limb of the incus. This connection occurred on the lateral aspect of the otic capsule and close to the horizontal course of the facial nerve. The incus was most likely to originate from the first pharyngeal arch, although the anlage was independent of Meckel's cartilage. This situation was similar to that of the stapes, which were derived from the second pharyngeal arch but independent of Reichert's cartilage (Rodríguez-Vázquez, 2005).

An inconsistent and unclear boundary between these two arches might support a theory stating that the handle of the malleus as well as the long limb of the incus would be derived partially from the second arch (Anson et al., 1960; Hanson et al., 1962; Strickland et al., 1962). However, our observation was consistent with results of recent molecular biology showing that the incus came from the first pharyngeal arch (Noden, 1983; Gendron-Maguire et al., 1993; Rijli et al., 1993; O′Gorman, 2005). The cranial end of Meckel's cartilage develops into the malleus except for its anterior process coming from the os goniale (Rodríguez-Vázquez et al., 1991). These results support a respective homology in evolution: the articular to the malleus, the quadratus to the incus and, the prearticular to the os goniale (Reichert, 1837; Gregory, 1913; Westoll, 1944, 1945; Hopson, 1966; Allin, 1975; Wang et al., 2001; Meng et al., 2011). In this context, the primary jaw joint is found between the

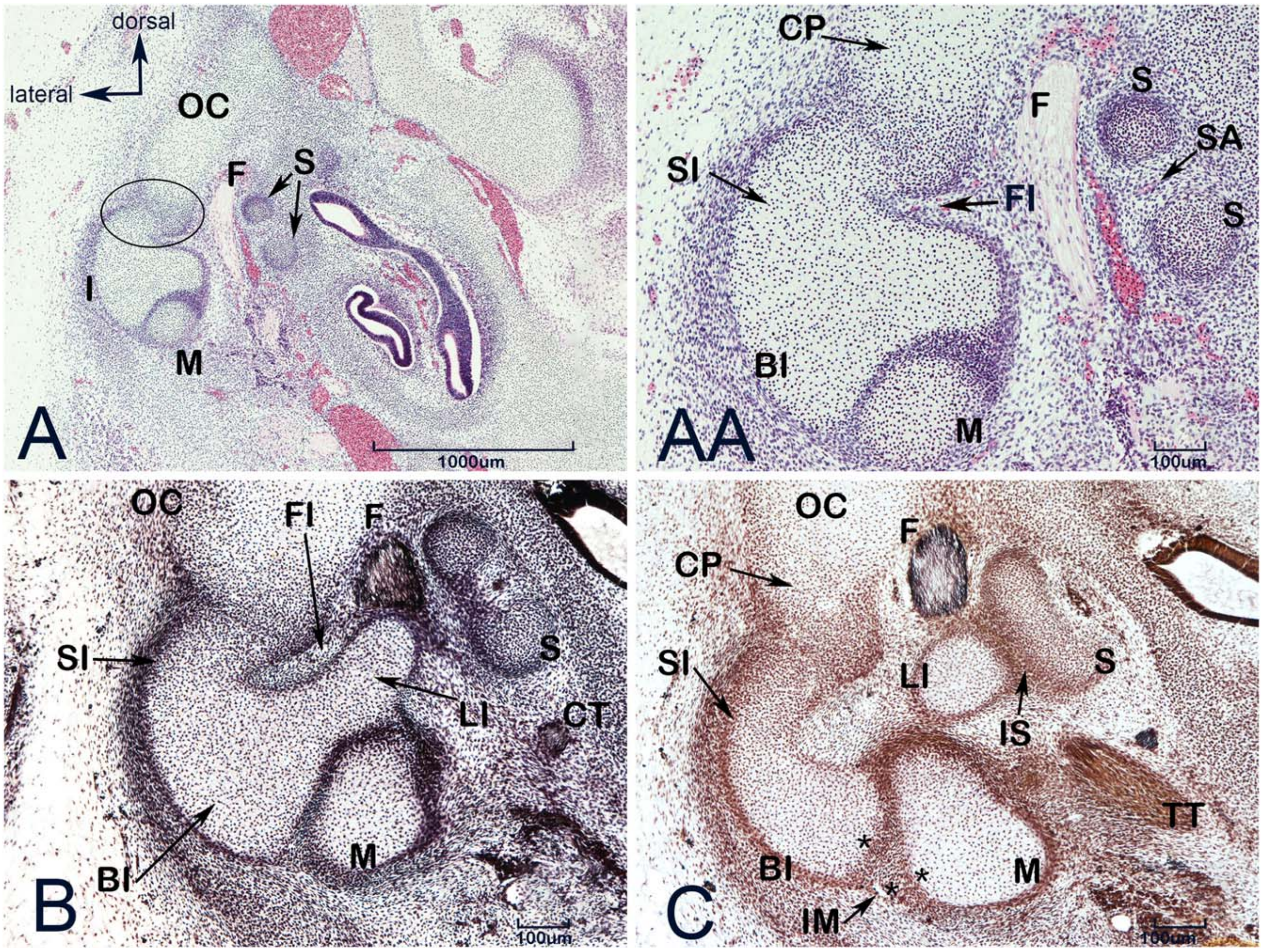

Fig. 5. Precartilaginous phase of the connection of the incus to the otic capsule. 7 WD. Human embryo of 21 mm GL (20 CS). Horizontal section (Panels A,AA). The Panel (AA) is an enlargement of Panel (A). Precartilaginous connection between the crista parotica (CP) and short limb of the incus (SI) formed an S. The formation of a "incudo-capsular fissure" (FI) between the otic capsule (OC) and the body of the incus (BI) starts. Human embryo of 23 mm GL (21 CS) Horizontal sections. Panel (B) is the most cranial level and panel (C) is the most caudal. The short limb of the incus (SI) connected with the crista parotica (CP) of the otic capsule (OC). A larger fossa (FI) was observed between the otic capsule and incus ("incudo-capsular fissure"). The incudomalleolar joint (IM) was in the trilaminar interzone (asterisks). CT, chorda tympani; F, facial nerve; I, incus; IS, incudostapedial joint; LI, long limb of the incus; M, malleus; S, stapes; SA stapedial artery; TT, tensor tympani muscle.

quadrate-articular (Gaupp, 1913). Does this correspond to the incudomalleolar joint in human?

Molecular biology can also help identify homologous elements. In Hoxa2 mutants, the second arch is transformed into a proximal first arch, while second-arch-derived structures, such as the stapes and Reichert's cartilage, are missing. In their place, an ectopic malleus, incus, and tympanic ring form (Gendron-Maguire et al., 1993). In addition, an ectopic cartilage was found connected to the incus, suggesting that it could be a palatoquadrate, which is homologous to the incus in primitive vertebrates (Rijli et al., 1993). Likewise, a similar ectopic palatoquadrate has been reported in Dlx2 mutants (Qiu et al., 1995). Such changes may cause the chondrification of the connective tissue thread that links the incus to the ala temporalis (Anthwal et al., 2013). These findings provide compelling evidence to support our results.

The second step at 7 and 8 WD was characterized by the cartilaginous incus (its short limb) connecting with the otic capsule at the crista parotica (Fig. 9B). Notably, the cranial end of Reichert's cartilage also joined to the otic capsule at the crista parotica. The third step at 9 WD was characterized by an initial detachment or disconnection of the incus from the otic capsule to provide an "incudocapsular" interzone. At 12 WD, a small cavity appeared within the "incudocapsular" interzone. At 13 WD, a cavity appeared between the otic capsule and the incus and was regarded as a syndesmosis joint (Fig. 9C). A joint between the incus and crista parotica is seen in foetal marsupials (Rowe, 1988; Luo, 1994; Luo and Crompton, 1994). Development of the human incus appears to be equivalent to that in marsupials (Sánchez-Villagra et al., 2002). During the evolution of mammals, the incudal articulation to the crista parotica preceded the detachment of the middle-ear ossicles from the mandible (Luo, 1994; Luo and Crompton, 1994). Our group has demonstrated a process of detachment of the malleus from Meckel's cartilage. A part of this cartilage

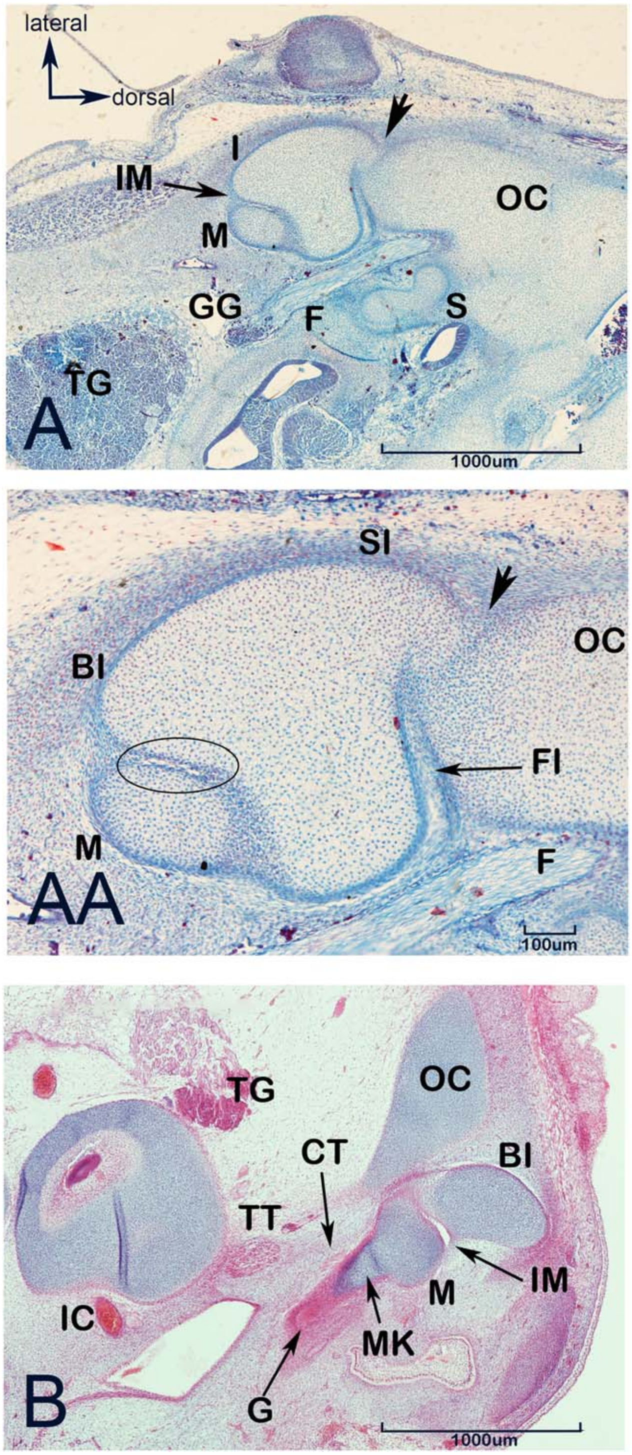

Fig. 6. Cartilaginous phase of the connection of the incus to the otic capsule. 8 WD. Human embryo of 26.5 mm GL (22 CS). Horizontal section (Panel A, AA). The Panel (AA) is an enlargement of Panel (A). Cartilaginous connection (arrow) between short limb of the incus (SI) and otic capsule (OC). A small cavity appeared in the interzone of the incudomalleolar joint (surrounded by oval) (Panel AA). Human embryo of 28 mm GL. Frontal sections (Panel B). The cavity of incudomalleolar joint (IM) becomes larger. The malleus (M) is continuous with Meckel's cartilage (MK). In this area, Meckel's cartilage (MK) is located cranially to the os goniale (G), and laterally to the chorda tympani (CT). BI, body of the incus; F, facial nerve; FI, incudo-capsular fissure; GG, geniculate ganglion; IC, internal carotid artery; TG, trigeminal ganglion; TT, tensor tympani muscle.

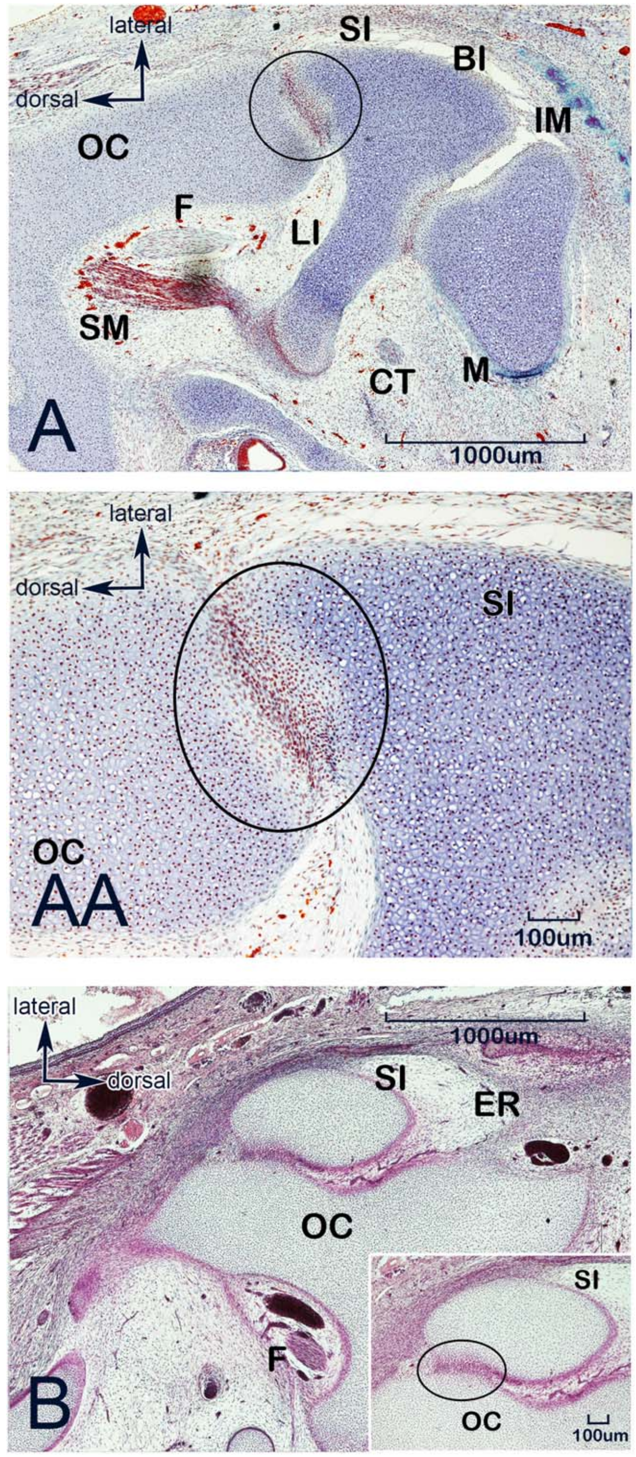

Fig. 7. Initial disconnection phase of the incus to the otic capsule. Formation of interzone between otic capsule and incus. 9–11 WD. Human foetus of 41.5 mm GL (9 WD). Horizontal section. (Panel A,AA). The Panel (AA) is an enlargement of Panel (A). Interzone (surrounded by oval) between the otic capsule (OC) and short limb of the incus (SI). The cavity of the incudomalleolar joint (IM) was larger than 8 WD. Human foetus of 62 mm GL (11 WD). Horizontal sections (Panel B). Interzone (surrounded by oval) between the otic capsule (OC) and the short limb of the incus (SI) was located dorsally to the facial canal and facial nerve (F), in the future fossa incudis of the epitympanic recess (ER). BI, body of the incus; CT, chorda tympani; LI, long limb of the incus; M, malleus; SM, stapedius muscle.

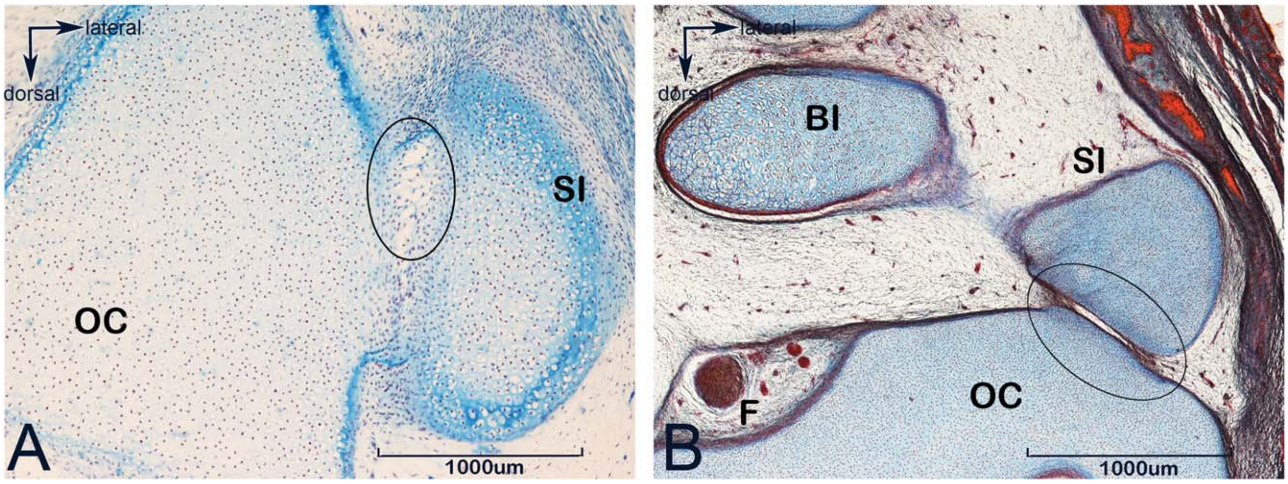

Fig. 8. Disconnection phase of the incus to the otic capsule. Formation of the joint between the otic capsule and the incus ("incudocapsular joint"). 12–13 WD. Human foetus of the 74.5 mm GL (12 WD). Horizontal section (Panel A). Small cavity (surrounded by oval) between the otic capsule (OC) and the short limb of the incus (SI). Human foetus of 100 mm GL (13 WD) (Panel B). Cavity between the short limb of the incus (SI) and the otic capsule (OC) (surrounded by oval). BI, body of the incus; F, facial nerve.

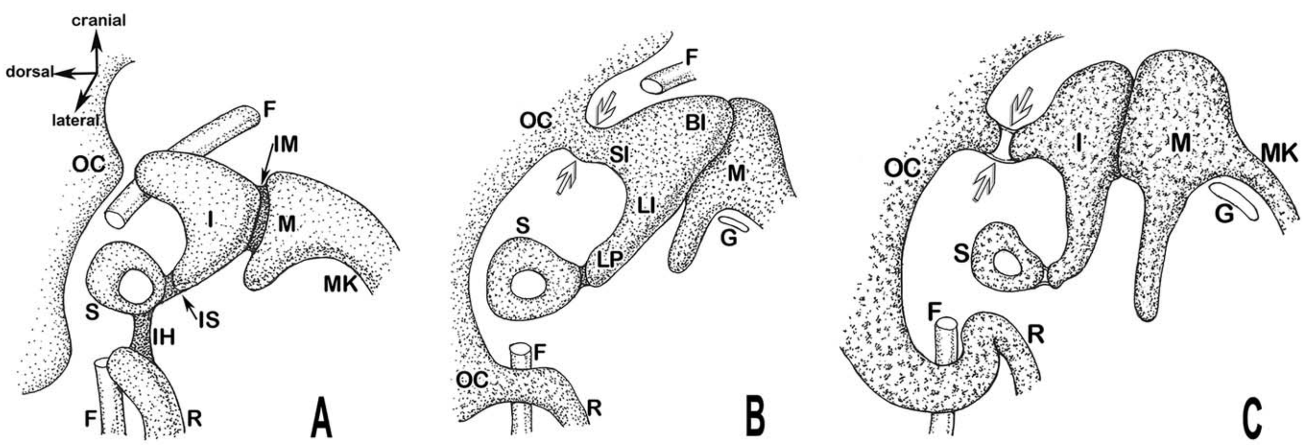

Fig. 9. Diagrammatic representation of the development of the incus and its relationship with the otic capsule. A.The anlage of the incus (I) was located close to the otic capsule. 6 WD. B. Precartilaginous and cartilaginous phase of the connection (arrow) of the short limb of the incus (SI) to the otic capsule (OC). 7–8 WD. C. Disconnection phase (arrows) of the incus (I) to the otic capsule (OC), 9–13 WD. BI, body of the incus; F, facial nerve; G, goniale; IH, interhyale; IM, interzone of the incudomalleolar joint; IS, interzone of the incudostapedial joint; LI, long limb of the incus; LP, lenticular process of the incus; M, malleus; MK, Meckel's cartilage; R, Reichert's cartilage; S, stapes.

transformed into the anterior ligament of the malleus as well as the sphenomandibular ligament, and both of the ligaments are continuous after closure of the middle ear (Rodríguez-Vázquez et al., 1992, 1993, 2011).

The cartilaginous connection between the incus and chondrocranium could allow the incus to be fixed against the first reflex movement at the mouth at 8–9 WD (Humphrey, 1968) via the incudomaleolar joint. At this stage, the temporomandibular joint is not yet formed (Mérida-Velasco et al., 1999). Therefore, this initial movement corresponds to a morphology of the primary jaw articulation. The incus was fixed to or provided a joint with the otic capsule, while the malleus was continuous with Meckel's cartilage. The latter is connected to the mandible by endochondral ossification (Rodríguez-Vázquez et al., 1997). Wang et al. (2001) found evidence of such a transitional state in fossils of early Cretaceous mammals. Likewise, in marsupial neonates (Kuhn, 1971; Maier, 1987), elasticity of the proximal portion of Meckel's cartilage most likely plays a mechanical role to avoid stress in the suckling. Our results provide significant evidence that the incus was derived in the first pharyngeal arch but independently from Meckel's cartilage, since the location of the incus anlage was different from that of the malleus-Meckel's anlagen. The transient attachment of the incus to the chondrocranium might support the incus-quadrate homology according to theories by Reichert (1837) and Gaupp (1913).

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