

Research article

Fetal development of the elastic-fiber-mediated enthesis in the human middle ear

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SUMMARY

In the human middle ear, the annular ligament of the incudostapedial joint and the insertions of the tensor tympani and stapedius muscles contain abundant elastic fibers; i.e., the elastic-fiber-mediated entheses. Hyaluronan also coexists with the elastic fibers. In the present study using immunohistochemistry, we demonstrated the distribution of elastin not only in the incudostapedial joint but also in the other two joints of the middle ear in adults and fetuses. In adults, the expression of elastin did not extend out of the annular ligament composed of mature elastic fibers but clearly overlapped with it. Electron microscopic observations of the annular ligament demonstrated a few microfibrils along the elastic fibers. Thus, in contrast to the vocal cord, the middle ear entheses seemed not to contain elastin and oxytalan fibers. In mid-term fetuses (at approximately 15–16 weeks of gestation) before opening of the external acoustic meatus, the incudostapedial joint showed abundant elastic fibers, but the incudomalleolar and stapediostapedial joints did not. At this stage, hyaluronan was not colocalized, but distributed diffusely in loose mesenchymal tissues surrounding the ear ossicles. Therefore, fetal development of elastin and elastic fibers in the middle ear entheses is unlikely to require acoustic oscillation. In late-stage fetuses (25–30 weeks), whose ear ossicles were almost the same size as those in adults, we observed bundling and branching of elastic fibers. However, hyaluronan expression was not as strong as in adults. Colocalization between elastic fibers and hyaluronan appeared to be a result of postnatal maturation of the entheses.

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1. Introduction

We recently reported unique entheses (bone–ligament and bone–tendon interfaces) in the human adult middle ear: the annular (collateral) ligament of the incudostapedial joint and the bony insertions of the tensor tympani and stapedius muscles are composed of elastic fibers rather than collagen fibers (Kawase et al., 2012). In the unique entheses that conduct acoustic oscillations,

mature elastic fibers, identified using both aldehyde–fuchsin and elastica–Masson stainings, were found to contain hyaluronan (hyaluronic acid), versican and fibronectin. Such colocalization of elastic elements and matrix substances, including elastin and hyaluronan, is well known to occur in the human vocal fold (Hammond et al., 1997; Hahn et al., 2006; Yamashita et al., 2010; Korn et al., 2011), although the vocal fold elastic elements are composed predominantly of elastin and oxytalan fibers (Hammond et al., 1997). The combination of elastin and hyaluronan has been considered an adaptation to vibration of the vocal fold.

The middle ear articulations include the incudomalleolar, incudostapedial and stapediostapedial joints. The incudomalleolar joint corresponds to the primary jaw of vertebrates (De la Cuadra Blanco et al., 2012) and, in fetal development, it is well known to form between the dorsal end of the first pharyngeal arch (Meckel's cartilage) and the incus (Rodríguez-Vázquez et al., 1991). The stapediostapedial joint provides an interface between the otic capsule

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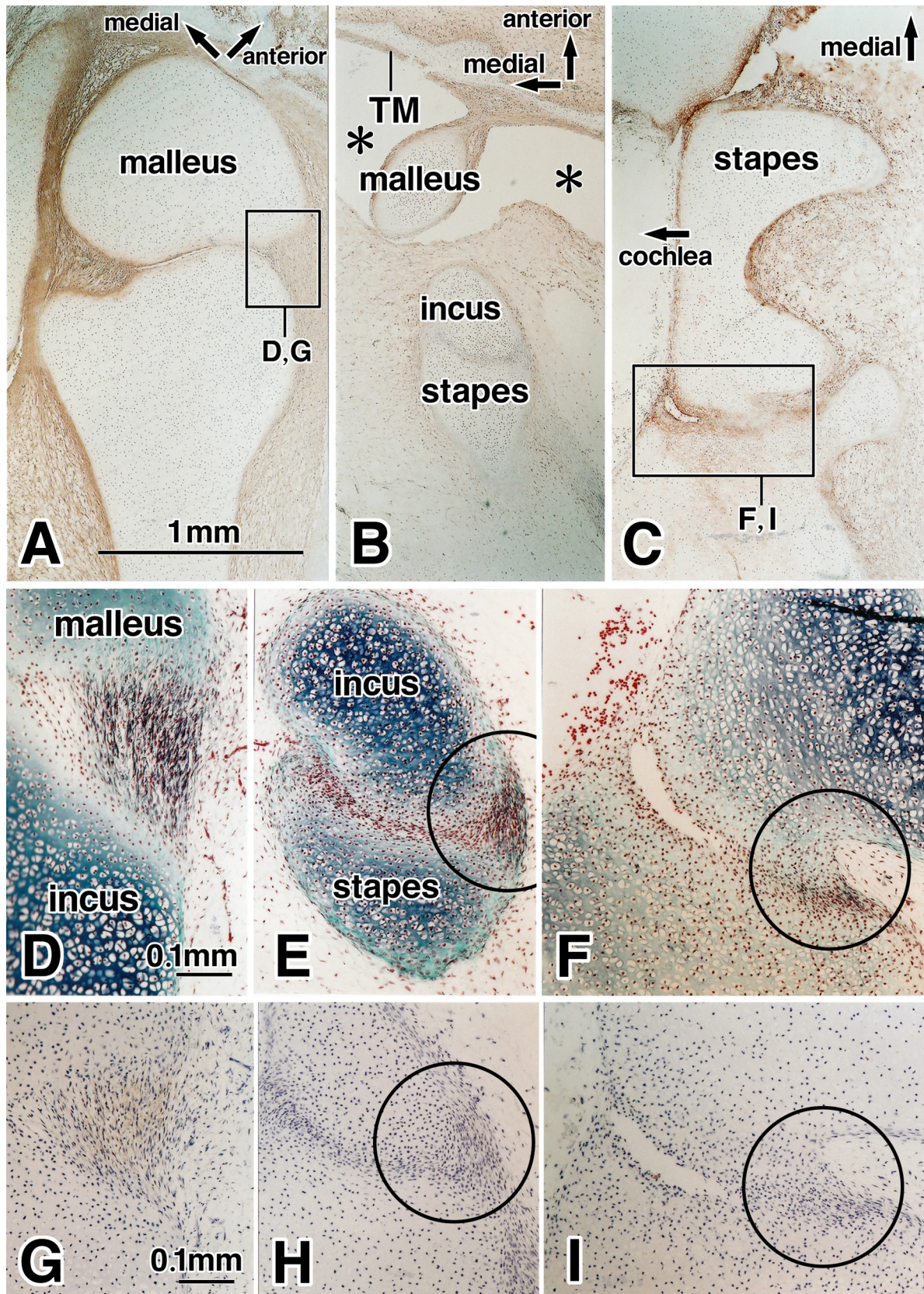


Fig. 1. Joints between ear ossicles in a mid-term fetus. All specimens were obtained from a 16-week male fetus. Panels A–C, hyaluronan staining; Panels D–F, elastica-Masson staining; Panels G–I, elastin immunostaining. Panels A, D and G (near sections) show the incudomalleolar joint, panels B, E and H (near sections) display the incudostapedial joint, while panels C, F and I (near sections) exhibit the stapediostapedial joint. Hyaluronan is diffusely positive in loose mesenchymal tissues around the ear ossicles (panels A–C). Black-colored elastic fibers are most evident in the incudomalleolar joint (panel D), moderately stained in the incudostapedial joint (circle in panel E) and weakly stained in the stapediostapedial joint (panel F). Likewise, elastin reactivity is relatively high in the incudomalleolar joint (panel G), weak in the incudostapedial joint (panel H) and faint or absent in the stapediostapedial joint (panel I). Asterisks in panel B indicate a cell-free space (the putative tympanic cavity) developing along the tympanic membrane (TM). Panels A–C (D–I) are prepared at the same magnification (scale bars in panels A, D and G). Counterstaining with hematoxylin was performed for the immunostaining.

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