THE REGISTRY

Newsletter of the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry

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MISSION STATEMENT

The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry was established in 1992 by the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health to continue and expand upon the former National Temporal Bone Banks Program. The Registry promotes research on hearing and balance disorders and serves as a resource for the public and scientific communities about research on the pathology of the human auditory and vestibular systems.

Cytomegalovirus-induced pathology in human temporal bones with congenital and aquired infection

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Cytomegalovirus (CMV) infection is one of the most frequent causes of sensorineural hearing loss at birth[1]. The mechanism of hearing loss caused by CMV infection has been thoroughly studied in animals, including neonatal mice, and seems to be mediated by the host immune response through the expression of pro-inflammatory chemokines[1]. In these animal models, the inner ears showed significant loss of hair cells and loss of spiral ganglion neurons. Viral antigens have been found in the organs of Corti, spiral ganglion, scala media and Reissner's membrane [2,3].

Although there have been numerous experimental studies on animals, human otopathological analyses of the cochleovestibular pathology of congenital and acquired CMV infections are less common. Therefore, the objective of this study was to investigate and compare auditory and vestibular histopathology in human temporal bones of newborns with congenital CMV infection and adults with acquired CMV infection. With the collected data, we aimed to better understand hearing loss and vestibular dysfunction in CMV-infected patients.

From the otopathology laboratories at Massachusetts Eye and Ear (Boston, MA) and the University of Minnesota (Minneapolis, MN), we selected human temporal bones from two deceased newborns with congenital CMV infection (Cases 1 and 2) and from two

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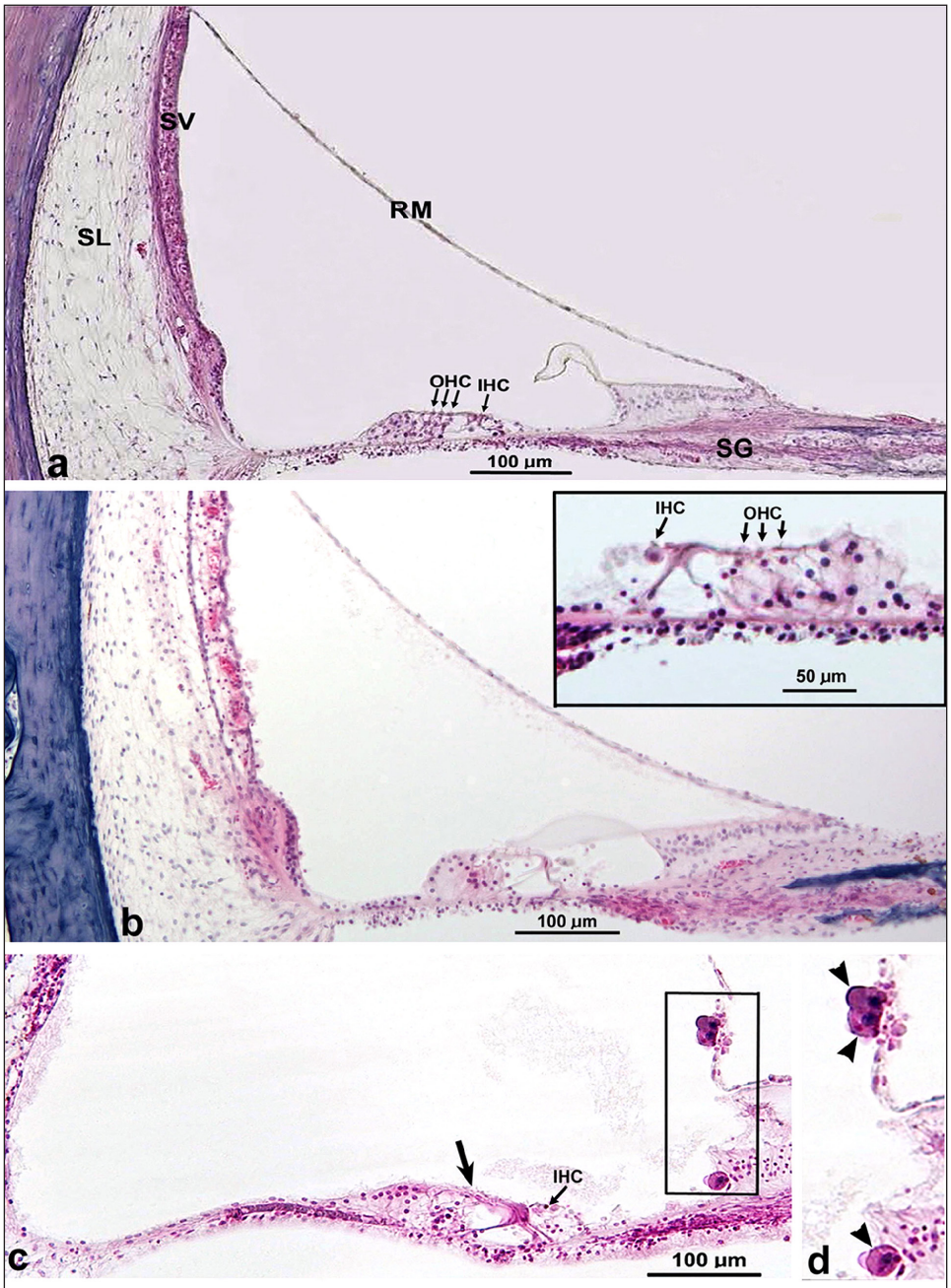

Figure 1. (a) Image of control temporal bone of a 2-day old boy. **(b)** Temporal bone of an 11-day old boy with congenital CMV infection (Case 1) shows hypervascularization and enlarged blood vessels in the stria vascularis with the intermediate layer of the stria vascularis most affected.

Organ of Corti shows outer and inner ear hair cells **(b, inset)**. **(c)** Temporal bone of a 3-week-old girl with congenital CMV infection (Case 2) shows loss of outer hair cells (arrow). **(d)** Enlarged view of the boxed area in **(c)**. Arrowheads indicate cytomegalic cells in the endolymphatic space of the cochlea. Hematoxylin and eosin staining. SV = stria vascularis, SL = spiral ligament, RM = Reissner's membrane, IHC = inner hair cells, OHC = outer hair cells, SG = spiral ganglion.

deceased adult donors with acquired virus infection (Cases 3 and 4). The temporal bones were harvested during autopsy, with postmortem time ranging between six and 14 hours. The temporal bones were processed using a standard protocol (formalin fixation, decalcification with ethylenediaminetetraacetic acid and embedded in celloidin). Serial sectioning was performed in the horizontal plane at a thickness of 20 µm. Every tenth section was stained with hematoxylin and eosin (H&E) and mounted on glass slides for light microscopic observation. To evaluate some types of

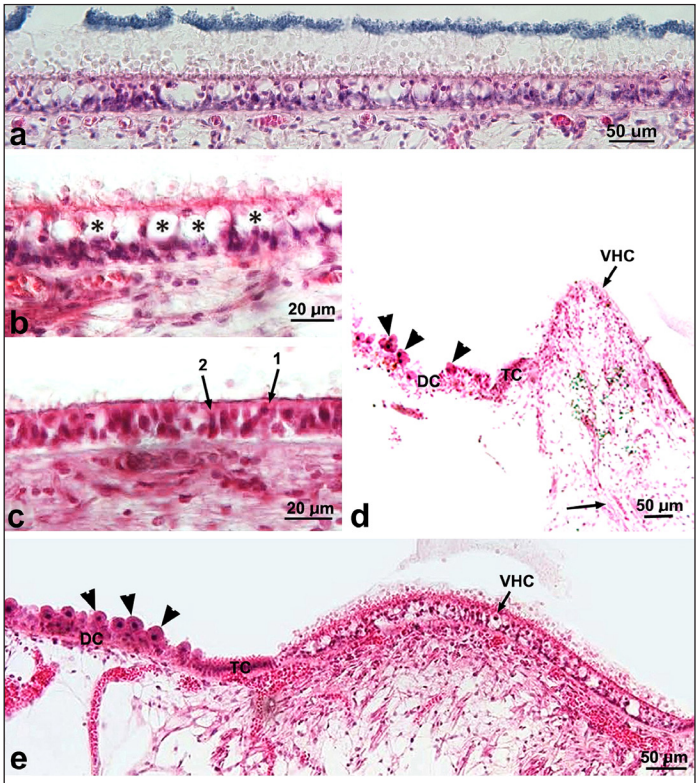

Figure 2. (a) Representative image of the maculae of utricle in human temporal bone with congenital CMV infection (Case 1) as seen by DIC microscopy. **(b)** Loss of type I and type II vestibular hair cells (asterisks) in the maculae of the saccule (Case 1). **(c)** Vestibular types I and type II hair cells (arrows) in normal temporal bone. **(c, d)** Loss of vestibular hair cells and degeneration of vestibular nerves (arrow) in the posterior semicircular canal (Case 2) **(d)** and in the lateral semicircular canal (Case 1) **(e)**. Cytomegalic cells (arrowheads) are located along the dark cells. Hematoxylin and eosin staining. VHC = vestibular hair cells, DC = dark cells, TC = transitional cells.

hair cells and other cells, we also used a differential interference contrast (DIC) microscopy. The Institutional Review Board of the University of Minnesota approved this study (0206M26181).

We characterized histological changes in the basilar, middle and apical cochlear turns for loss of hair cells and neurofilaments, abnormalities of the stria vascularis and spiral ligament and the presence of cytomegalic cells with CMV inclusion bodies. In the peripheral vestibular system, we analyzed the loss of vestibular hair cells, degeneration of vestibular nerves and presence of cytomegalic cells. We also selected age-matched controls for the temporal bones with CMV. The infant CMV cases were compared with nondiseased infant bones. Both adult donors with acquired CMV infection had leukemia, and thus we compared these cases to temporal bones of age-matched donors without CMV infection and leukemia.

Otopathologic analysis of the infant temporal bones from the control group did not reveal abnormalities affecting their middle ears. Their cochlea had intact inner and outer hair cells. No atrophy of the stria vascularis or loss of fibrocytes in the spiral ligament was observed, and the sensory vestibular epithelium was within normal limits. The temporal bones of the two newborns with congenital CMV infection (Cases 1 and 2) had several abnormalities: Their middle ears were filled with abundant inflammatory cells (monocytes and neutrophils) and showed signs of fibrosis. In the inner ear, CMV-infected temporal bones showed hypervascularization and enlarged blood vessels in the stria vascularis in all cochlear turns (Fig. 1), as well as cellular loss and edema. Case 2 showed some loss of outer cochlear hair cells (Fig. 1c, arrow), while Case 1 had intact organs of Corti. In Case 2, we found large "owls-eye" cytomegalic cells with CMV inclusion bodies in the cochlear duct, including cells adjacent to Reissner's membrane (Fig. 1c, d). No cytomegalic cells were found in the organ of Corti. In the sensory vestibular epithelium, the CMV-infected temporal bones showed loss of type I and type II hair cells in the macula of the utricle (Fig. 2a) and saccule (Fig. 2b) as compared to normal temporal bones (Fig. 2c). There was also loss of vestibular hair cells and degeneration of vestibular nerves in the semicircular canals (Fig. 2d, e). Cytomegalic cells were found mostly on the dark cells in the semicircular canals and utricle of Case 1 (Fig. 2d) and Case 2 (Fig. 2e). Fewer cytomegalic cells were on the wall and in the epithelium of the vestibular labyrinth. The temporal bones from adult donors who had acquired CMV infection (Cases 3 and 4) did not show significant abnormalities as compared with controls. The middle ears of Cases 3 and 4 were normal. In the cochlea, both outer and inner hair cells seemed structurally normal. There was mild atrophy of the stria vascularis in the middle turn of the cochlea (Case 4) and loss of fibrocytes in the spiral ligament, which was more noticeable for type I and type II fibrocytes in both donors. Some melanin-like pigmentation was observed in the stria vascularis. The sensory epithelium of the vestibular system did not show loss of hair cells or other structural abnormalities in either case of acquired CMV infection. No evidence of cytomegalic cells was found in the cochlea or vestibular membranous labyrinth.

To our knowledge, our study was the first to evaluate and compare the otopathologic findings in human temporal bones from donors with congenital and acquired CMV. Our findings revealed several abnormal findings in the middle ears and the inner ears of infants with congenital CMV. The presence of middle ear inflammation has been reported in clinical studies: Chonmaitree et al.[4] found that about 10 percent of infants and children with acute otitis media were also infected with CMV or herpes simplex virus type 1. The cochlear changes we

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observed (loss of hair cells strial atrophy) are consistent with reported clinical records of sensorineural hearing loss in infants and young children with congenital CMV infection[5-7]. We found cytomegalic cells on the Reissner's membrane and in other sites of the cochlear endolymph in infected newborns; however, they were not observed in the organ of Corti. More cytomegalic cells were seen in the vestibular system compared to the cochlea. Some of the dark cells in contact or vicinity of cytomegalic cells appear to be damaged (Fig. 2d, e). Our data is consistent with a high concentration of cytomegalic cells in the regions of dark cells in the utricle found by Davis et al.[8]. The presence of cytomegalic cells may be related to a high viral load leading to loss of vestibular hair cells in the macula of saccule and utricle and to loss of hair cells and/or degeneration of vestibular nerves in the semicircular canals. In fact, the infants with congenital CMV (Cases 1 and 2) showed a higher damage to hair cells and other inner ear structures in areas having cytomegalic cells, probably due to direct cytophatic effect of the virus. The vestibular hair cells are involved in mechano-electrical transduction[9], while the dark cells are responsible for endolymph secretion[7,10]. Both types of cells and vestibular nerves were affected in temporal bones with congenital infection, explaining the symptoms of balance disorders and dizziness that are frequent in children with congenital CMV infection[11]. Partial and bilateral vestibular impairment were found in 43 percent[12] and balance disorders in 90 percent[13] of these children.

None of the adult donors with acquired CMV infection (Cases 3 and 4) had a history of hearing loss or vestibular dysfunction. The temporal bones showed little or no pathology, consistent with reports that most children and adults who are diagnosed with acquired CMV do not develop clinical symptoms. Cochlear and vestibular hair cells of adult donors were intact, but we observed some melanin-like pigmentation in the stria vascularis. We have seen similar pigment granules in human temporal bones from age-matched adult donors with leukemia, but without CMV infection. Melanin-bearing cells of the stria vascularis have been reported to show increased activity in response to infection, noise and harmful substances[14,15]; however, the exact meaning of this finding in these temporal bones with acquired CMV still remains to be clarified.

In conclusion, the histopathological changes were more severe in the congenitally infected group compared to those in the acquired infection group. In infants with congenital infection, cytomegalic cells were mostly seen in vestibular organs. Their cochlea, vestibular system and middle ear were highly affected, which might be the pathologic basis of clinically encountered auditory and vestibular disorders in those patients.

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Developmental anomalies of the human stapes

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Conductive hearing loss can be the result of congenital hearing bone (ossicle) malformations that are estimated to be present in one per 15,000 births[1]. The stapes is the smallest bone in the human body. It is shaped like a stirrup and conducts sound vibrations from the incus to the inner ear. It is composed of a head or capitulum, which articulates with the incus through the incudo-stapedial joint, and two limbs, or crura, that connect to a footplate. The footplate is the base of the stapes that sits in the oval window connected to the otic capsule by an annular ligament. The annular ligament allows vibration of the footplate thereby transfering sound to the scala vestibuli. Congenital malformations of the stapes can result in conductive hearing loss.

Recent animal studies[2-9] have begun to shed light on how the ossicles—including the third bone of hearing, the stapes—are formed. The stapes can be divided into anatomic subunits including the capitulum, crura and inner footplate that are of neural crest origin, and the outer footplate, annulus and oval window that are of mesodermal origin[5,9]. We recently completed a study to determine if human stapes abnormalities can be explained by disruptions in the known dual neural crest and mesoderm origin of the stapes.

In order to conduct this study, we identified temporal bones with congenital ossicular anomalies in the Massachusetts Eye and Ear temporal bone collection. The clinical history was collected during life through enrollment in the National Institute on Deafness and Communication Disorders (NIDCD) National Temporal Bone, Hearing and Balance Pathology Resource Registry.

Each case could be classified into one of four malformation types based on our current understanding of the embryologic origin of the subunits of the stapes and timing of development. Figure 1 shows a representative example of each malformation by type.

In the first pattern, an early disruption of neural crest cell derived stapes subunits affects the development of the mesoderm derived outer footplate, annular ligament and oval window. In the second, there are more restricted developmental anomalies of the neural crest and mesodermal derived subunits. In this second group, the capitulum and crura are dysmorphic or monopodal and, while the footplate may be fixed, the footplate and oval window are otherwise developed. The third and fourth types of malformations exhibit a more restricted dysmorphology, which suggests—albeit not conclusively—the disruption of events occurring later in stapes development than seen in the first and second group. The third group consists of anomalies affecting the endochondral ossification of the neural crest derived portion of the stapes including capitulum, crura and inner footplate. Finally, the fourth and most common category is isolated fixation of the stapes. This points to disruption of an event restricted to the mesodermal derived annular ligament.

Malformations of the human stapes follow consistent patterns of early or late disruptions of the stapes subunits of mesodermal and/or neural crest origin. This categorization can serve to inform radiographic interpretations of stapes morphology. Recognition of anomalies affecting both the neural crest and mesoderm derived subunits point to more

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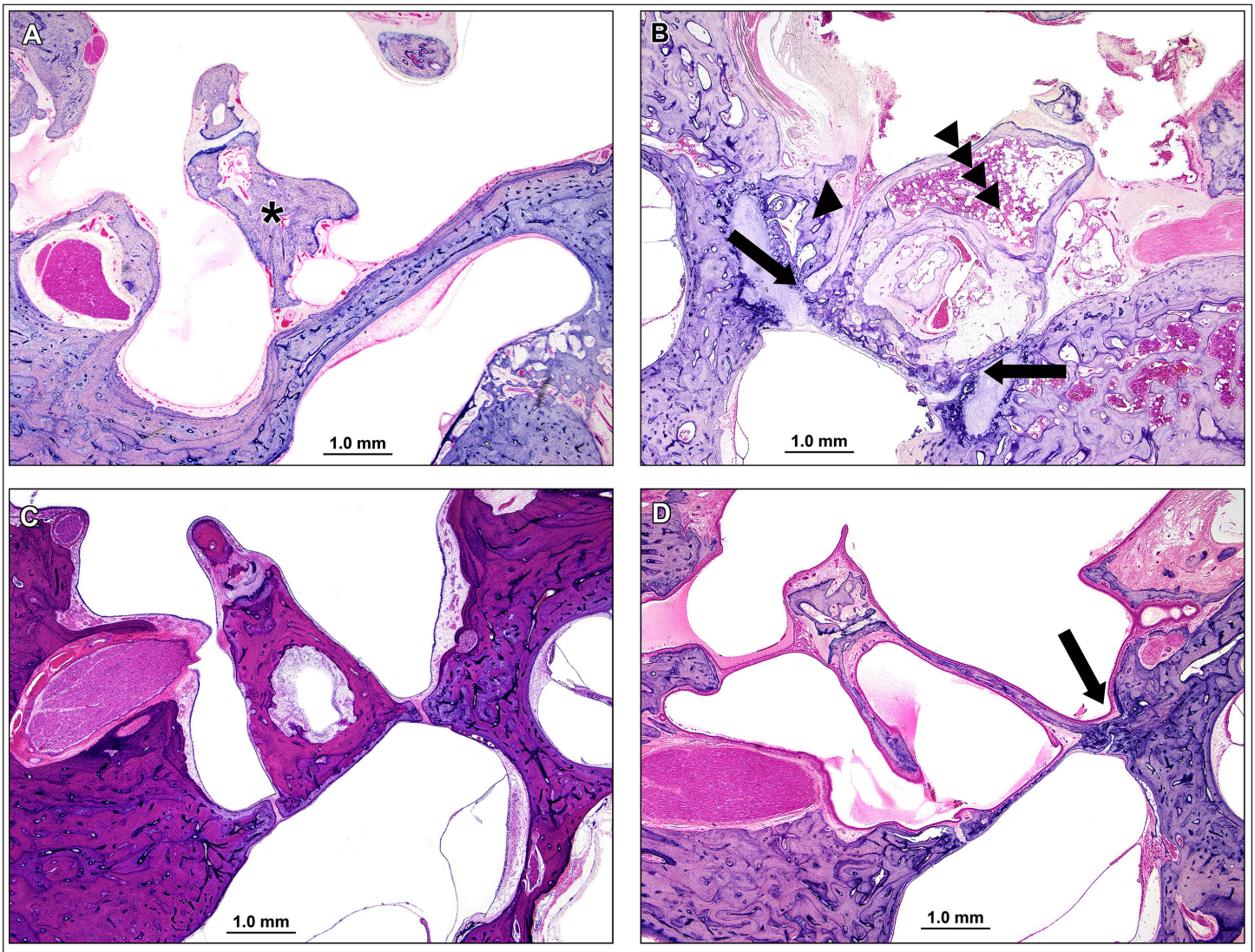

Figure 1 a-d. Representative sections of each malformation by type. **(a)** Type I: There is no footplate or oval window. The crura and capitulum are monopodal (asterix) **(b)** Type II: Dysmorphic capitulum and crura (arrowheads) and a fixed footplate (arrow). The the oval window is present. **(c)** Type III: The footplate and oval window are present and without fixation. The stapes crura and capitulum are thick and dysmorphic. **(d)** Type IV: Isolated fixation of the stapes footplate (arrow).

global disruptions and, in turn, isolated footplate fixations point to a more restricted process that may be amenable to surgical correction. While the molecular events, including temporal coordination, that lead to a normally formed stapes are not yet fully understood, the observed patterns of human stapes malformation can be consistently classified into one of four patterns of developmental disruption.

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