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FOCAL DEGENERATION OF VESTIBULAR NEUROEPITHELIUM IN THE CRISTAE AMPULLARES OF THREE HUMAN SUBJECTS

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Abstract

Background: We report a unique pattern of focal degeneration of the neuroepithelium of cristae ampullares, thick subepithelial extracellular deposits, and neural degeneration in three humans.

Objective: To characterize the pattern of vestibular degeneration and measure the thickness of subepithelial deposits in these three cases and controls.

Methods: The subepithelial deposits of vestibular end organs in three subject cases and controls were studied using H&E, PAS, Gomori trichrome staining, and immunostaining for anti-neurofilament, anti-myosin VIIa, and anti-collagen 4a1. The thickness of deposit as measured by light microscopy was compared to that of control groups (age-matched controls, patients with unilateral Meniere's disease, vestibular neuritis, cupulolithiasis, severe non-focal degeneration of the vestibular neuroepithelium, and Alport syndrome). The correlation of thickness of deposits with age from 0–100 years was also investigated.

Results: Focal loss of hair cells in the neuroepithelium, thick subepithelial deposits, and degeneration of subepithelial dendrites and Scarpa's ganglion were found in all three cristae of three subject cases. Immunostaining demonstrated a decrease of afferent neural fibers in the cristae and focal fragmentation of the basement membrane adjacent to the deposits. The thickness of the subepithelial deposits in three cristae of three subject cases was significantly greater than that of all controls. In the three cristae of normal controls, the thickness of deposits demonstrated a positive correlation with age.

Conclusion: Although both age and degeneration of the vestibular neuroepithelium may be associated with the thickness of the subepithelial deposits, in this unique pattern of degeneration, the thickness of the subepithelial deposits was significantly greater than that in all controls.

Keywords

crista ampullaris; vestibular neuroepithelial degeneration; human temporal bone

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Introduction

The mammalian peripheral vestibular sensory system consists of the saccule, the utricle, and 3 semicircular canals. Each end organ contains neuroepithelium (macula sacculi, macula utriculi, and 3 cristae ampullares). The neuroepithelium is similar in all five end organs and consists of type 1 and type 2 hair cells with stereocilia, supporting cells, and innervating neurons via the superior and inferior vestibular nerve segments of the 8th cranial nerve (1).

The histopathologic correlate of peripheral vestibular dysfunction in many clinical disorders consists of atrophy of the components of vestibular neuroepithelium and atrophy of innervating vestibular neurons (2). We describe what appears to be a unique pattern of degeneration of the vestibular neuroepithelium in three human cases. It consisted of focal degeneration of the neuroepithelial sensory cells especially at the summit of all three cristae, a thick subjacent deposit of amorphous extracellular material, and atrophy of neuronal processes and Scarpa's ganglion. In each of the 3 cases there was a history of dizziness or possible vestibular symptoms. In addition we compared the histopathologic findings described in these three cases with age-matched controls with no vestibular symptoms, unilateral Meniere's disease, vestibular neuritis, cupulolithiasis, severe non-focal vestibular neuroepithelial degeneration, and Alport syndrome, in which abnormalities of basement membranes have been described (3, 4).

Materials and Methods

Selection of cases

The three subject cases were identified by search of existing digital images of the cristae ampullares in temporal bones of individuals who in life had symptoms possibly attributed to vestibular disease. Controls were identified by search of the database of the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry for specific diagnoses of vestibular disease. The demographics of the three subject cases studied are presented in Table 1A.

The thickness of the subepithelial deposit in the cristae and maculae of the three subject cases (n=6, mean 87.5 yrs.) was compared to age-matched controls (n=22, mean 87.2 yrs.), patients with unilateral Meniere's disease (n=9), unilateral vestibular neuritis (n=7), unilateral cupulolithiasis (n=10), severe non-focal degeneration of the vestibular neuroepithelium (n=14) and Alport syndrome (n=8) in order to evaluate the effect of aging, or other disorders. To further evaluate the effect of aging, a total of 111 specimens age 0–100 years and without a history of vertigo or dizziness during life were randomly selected for study.

Histologic Techniques

All temporal bones were removed and fixed in 10% buffered formalin. After decalcification in ethylene/diamine tetra-acetic acid (EDTA), the specimens were dehydrated in graded alcohols, embedded in celloidin, and sectioned at a thickness of 20 μ m in the axial (horizontal) plane. Every tenth section was stained with hematoxylin and eosin and mounted

on glass slides for study using light microscopy and Nomarski (differential interference contrast) microscopy. Adjacent sections were selected for special staining (PAS, Gomori trichrome, and immunostaining).

2-D reconstruction of the cochlea

Every tenth section was used to create a 2-dimensional reconstruction of the cochlea using proprietary software to document atrophy of spiral and Scarpa's ganglion cells, cochlear hair cells, and the stria vascularis.

Other staining techniques

Selected adjacent sections from the three cristae in the three subject cases were used for PAS staining, staining with Gomori trichrome, and immunostaining for anti-neurofilament, anti-myosin VIIa, and anti-collagen 4a1.

Immunohistochemical Methods

Immunohistochemical labeling of nerve fibers, vestibular hair cells, and collagen was accomplished in the three subject cases and in three control cases. Sections of the three subject specimens were selected for immunostaining in areas where the vestibular neuroepithelium had degenerated to a monolayer of cells. Control specimens were selected in areas that best matched the level of sectioning for the subject cases.

Immunolabeling of nerve fibers was accomplished using a mouse monoclonal primary antibody made against 200kD neurofilament (Boehringer Mannheim, Mannheim, Germany) at a dilution of 1:10,000. The anti-neurofilament antibody stains nerve fibers and their endings (5–7).

Labeling of the vestibular sensory cells was done with a rabbit polyclonal primary antibody made against myosin VIIa (ProteusBiosciences Inc., Ramona, CA) at a dilution of 1:100. Myosin VIIa is expressed in vestibular hair cells of both mice and humans (8, 9).

For the collagen 4a1 labeling, a mouse monoclonal antibody made against collagen IV alpha 1, clone 1043 (Novus Biologicals, Littleton, CO) was used at a dilution of 1:400 or 1:500. Collagen 4a1 has been described as being present in the human vestibular basement membrane (10, 11).

For all of the immunolabeling, the celloidin was first removed from sections using a solution of sodium hydroxide and methanol as previously described (5, 12). Primary antibodies were applied overnight and left in a humid chamber. Rinses of phosphate buffered saline (PBS) followed. Secondary antibodies made in the appropriate host species were applied at a dilution of 1:200 and left for 1 hour, then rinsed in PBS. Avidin-Biotin-horseradish peroxidase (standard ABC kit, Vector Labs, Burlingame, CA) was applied for 1 hour and then rinsed. Colorization was completed with 0.01% diaminobenzidine and 0.01% hydrogen peroxide (H₂O₂) for 5 to 10 minutes. Slides were rinsed in PBS, counterstained lightly with hematoxylin, and dehydrated in graded ethanols. Cover slips were applied (12).

Measurements of subepithelial amorphous extracellular material

The thickness of the subepithelial extracellular deposit was measured using Excelis HD imaging software (AccuScope, Inc., Commack, NY), coupled to an Olympus BX51 light microscope and imaging software (CAPTA Vision: AccuScope Inc.) to capture the images at 40× (1920 × 1080 pixels). All axial (horizontal) sections through the superior (n = 3–5), horizontal (n = 3–7), and posterior (n = 3–7) cristae, macula utriculi (n = 3–8), and macula sacculi (n = 5–11) were examined and measured in the three study cases and all controls. The maximum thickness of the subepithelial extracellular deposit was measured and the average maximum thickness of the subepithelial deposit was calculated for each end organ. Sections in which the boundary between extracellular deposit and stroma could not be clearly identified were excluded.

Statistical analysis

Measurements of the average maximum thickness of the deposit were statistically compared using the Mann-Whitney U test. To evaluate the correlation of age with thickness of the deposit, Spearman's correlation coefficient by rank test was used (GraphPad Prism version 7.02; GraphPad Software, Inc., LaJolla, CA).

Results

Case histories and cochlear histopathology

Case 1

OTOLOGIC HISTORY: This 89-year old man had three successive episodes of poorly defined dizziness at age 26, 35 and 52. Each episode lasted approximately one week. Audiograms at age 60, 78, 82, and 88 (Fig. 1) showed a bilateral downsloping sensorineural hearing loss which was progressive. Speech discrimination was relatively stable throughout this period. He wore hearing aids intermittently. There was no change in his hearing during his episodes of dizziness, and there was no tinnitus reported. He had a history of severe noise exposure as a pilot in WWII. No further diagnostics for his suspected vestibular disorder were done, and clinically he was suspected to have Meniere's syndrome, although the lack of history of true vertigo and fluctuating auditory symptom was not consistent with this diagnosis. His past medical history included squamous cell carcinoma of the left neck, chronic atrial fibrillation, gout, an abdominal aortic aneurysm, and hepatitis B. He had peripheral neuropathy of his legs, chronic renal insufficiency and renal cysts, with a BUN (blood urea nitrogen) of 35mg/dL and a creatinine of 2.1 mg/dL. He died of aspiration pneumonitis.

HISTOPATHOLOGY:

Right and Left cochleae: The histologic correlates of sensorineural hearing loss were degeneration of spiral ganglion cells, and atrophy of outer hair cells, particularly in the basal turn, and moderate degeneration of the stria vascularis in the apical one half of the cochlea. There was no evidence of endolymphatic hydrops. Cytocochleograms of both ears are shown in Figure 1.

The auditory nerve was partially avulsed from the internal auditory on the right. There was an arachnoid cyst in the internal auditory canal of the left ear located between the vestibular and facial nerves.

Case 2

OTOLOGIC HISTORY: This 89-year old female suffered from a chronic imbalance disorder and bilateral sensorineural hearing loss. Balance complaints began in her mid to late 70s, and she used a cane to walk for several years. In addition she reported three attacks of vertigo during this period, without change in hearing or tinnitus. A CT scan of the head at age 79 was negative. At the age 80, a question of a transient ischemic attack was raised. A carotid Doppler test demonstrated that both carotid arteries had normal blood flow. Flow in the left vertebral artery was not visualized. The cause of her balance disorder was attributed to possible benign positional vertigo, labyrinthitis, or vertebrobasilar insufficiency.

Hearing loss began in both ears in her early 40's following a single episode of acoustic trauma. Two years later she began to use a hearing aid for telephone communication. An audiogram performed at age 83 (Fig. 1) demonstrated a bilateral downsloping sensorineural hearing loss. Speech discrimination was 92% on the left and 76% on the right.

Past medical history included degenerative disc disease of the cervical spine, arthritis of the right hand, hypertension, hypocholesterolemia, dementia, and a systolic murmur. The cause of death was hemorrhagic stroke.

HISTOPATHOLOGY:

Right and Left Cochleae: The histologic correlates of hearing loss were atrophy of the stria vascularis, scattered loss of inner and outer hair cells, particularly in the basal turn, and cochlear neuronal atrophy, particularly in the basal turn. Cytocochleograms of both ears are shown in Figure 1.

Case 3.

OTOLOGIC HISTORY: This 82-year old man was evaluated at the age of 67 years for difficulty understanding speech in adverse listening conditions and poorly defined dizziness and tinnitus. An audiogram at age 67 (Fig. 1) demonstrated bilateral high tone sensorineural loss with 92% speech discrimination in both ears.

Past medical history included epileptic seizures for which he underwent resection of the hippocampus and adjacent structures of the middle temporal lobe bilaterally. This resulted in reduction of the frequency of seizures but with the complication of anterograde amnesia. He also had hypertension, bilateral emphysema, and vertebral osteoporosis, generalized severe atherosclerosis involving thoracic and abdominal vessels, cardiomegaly, bilateral nodular glomerulosclerosis and nephrosclerosis. The cause of death was aspiration pneumonia.

HISTOPATHOLOGY:

Right and Left Cochleae: Histopathologic correlates of the sensorineural loss included partial loss of both inner and outer hair cells, partial atrophy of the stria vascularis

throughout the cochlea, and moderate loss of spiral ganglion cells. Cytocochleograms of both ears are shown in Figure 1.

VESTIBULAR HISTOPATHOLOGY: (All three cases): There was no evidence of endolymphatic hydrops or cupulolithiasis in any case. The histopathology seen in all 3 cases included focal atrophy of the neuroepithelium of the cristae, atrophy of the dendrites of the vestibular nerves, decreased Scarpa's ganglion counts, and deposition of a thick layer of subepithelial acellular material (Fig. 2).

Focal atrophy of neuroepithelium of cristae

There was focal atrophy of the neuroepithelium at the summit of all three cristae ampullares in all three cases. In contrast, the macula utriculi and macula sacculi showed only scattered atrophy of the neuroepithelium. In the area of the summit of the cristae the thickness of the neuroepithelium was reduced with loss of hair cells resulting in a monolayer of predominantly supporting cells. Loss of hair cells at the summit was confirmed by myosin VIIa immunostaining (Fig. 3A, B). Scarpa's ganglion cell counts were reduced to approximately 60 to 70% of normal age-matched controls (13), (Table 1A). In addition, as shown by anti-neurofilament immunostaining, the numbers of neurons penetrating the subepithelial mesenchyme of the cristae and neurons radiating parallel to the neuroepithelial cells of the cristae were reduced compared to normal controls (Fig. 3C, D).

Histology of the subepithelial amorphous extracellular deposit

In all three cristae of the six temporal bones, there was a subepithelial deposit of amorphous extracellular material which was much thicker than in the macula utriculi or macula sacculi (Table 1A). The subepithelial deposit was thickest in the area of the summit of each crista (Fig. 2). The subepithelial deposit stained with PAS (periodic acid-Schiff) stain suggesting the presence of polysaccharide components within the subepithelial deposit (Fig. 3E). Gomori trichrome stain uniformly stained the subepithelial deposit blue suggesting the presence of collagen (Fig. 3F).

Immunostaining using anti-col 4A1 clearly delineated the presence of an intact basement membrane except at the summit of the cristae where the basement membrane appeared to become fragmented (Fig. 3G).

Thickness of the Subepithelial Deposit

The average maximum thickness of the subepithelial deposit is shown in Table 1A for the three study cases. The mean thickness of the subepithelial deposit of the three subject cases and all controls is displayed with p values in Figure 4A. The thickness of the subepithelial deposit in the cristae of the three subject cases was consistently greater than that of the age-matched control and the other controls. For the macula sacculi the thickness of the subepithelial deposit in the three subject cases was thicker than that in the age-matched controls and thinner than that of the three cristae. For the macula utriculi, the thickness of the subepithelial deposit tended to be thicker than that of age-matched controls but did not reach statistical significant ($p=0.07$) and thinner than that of the three cristae.

Comparison of the Thickness of the Subepithelial Deposit between diseased and normal ears

To evaluate the effect of the presence of various forms of vestibular disease on the thickness of the subepithelial deposit, a comparison of thickness between the diseased ears and the opposite unaffected ears or age-match control ears is displayed in (Fig. 4B). There was no significant difference in the thickness of the subepithelial deposit in ears affected by Meniere's disease, vestibular neuritis and cupulolithiasis as compared to the unaffected opposite side with the exception of the lateral semicircular canal where the thickness was significantly greater on the unaffected side in Meniere's disease. The thickness of the subepithelial deposit in the severe non-focal degeneration of the vestibular neuroepithelium was significantly thicker for two cristae ampullares and two maculae than the age-matched controls. The thickness of the subepithelial deposit in Alport syndrome was significantly thicker for two maculae than age-matched controls.

Correlation between thickness of the subepithelial deposit and age

The mean maximal thickness of the subepithelial deposit in each decade of life from age 0–100 years (n=111) is displayed in Table 1B. The thickness of the subepithelial deposit tended to increase with advancing age. To further evaluate the effect of age on the thickness of the subepithelial deposit a comparison of thicknesses from age 0–100 years was done (Fig. 5). These patients had no vestibular symptoms. Spearman's correlation coefficient by rank test demonstrated a significant positive correlation between age and thickness of the subepithelial deposit in the 3 cristae but not in the maculae utriculi or sacculi.

DISCUSSION

The combination of histopathologic findings in these three subject cases, demonstrating focal degeneration of the vestibular neuroepithelium of the cristae, atrophy of the dendrites of the vestibular nerve, decreased numbers of Scarpa's ganglion cells, and a thick subepithelial extracellular amorphous deposit appears to be unique and not previously reported. Although a subepithelial deposit was also seen in the aging cohort with no known vestibular symptoms, and in those with other vestibular disorders, (Meniere's disease, vestibular neuritis, cupulolithiasis, severe vestibular end organ degeneration) and in Alport syndrome, the thickness of the subepithelial deposit found in the cristae of the three subject cases was significantly greater than was found in all control cases.

Some of the findings in these subject cases are similar to the increase in thickness of the basement membrane of the vestibular end organs described in Meniere's disease (14), in the utricle from a patient with Meniere's disease (15), and in the endolymphatic sac in a Meniere's patient (16). In the report by McCall et al. (14), there were other similarities including a monolayer of degenerated neuroepithelium associated with thickening of the basement membrane. However, the basement membrane was 1.9 to 4.7 μm in thickness as reported by McCall et al. (14) compared to thickening of the subepithelial deposit up to 35 μm in the subject cases reported herein. The evidence obtained from immunostaining for anti-collagen col4A1 suggests that the thick subepithelial deposit described here was not limited to the basement membrane itself but included both the basement membrane and the

deposited amorphous material. Therefore, the subepithelial deposit in these three subject cases is not identical to the thickened basement membrane described in Meniere's disease by McCall et al. (14).

Since a thickened deposit was present bilaterally and in all three cristae, age was considered as a possible causative factor. A subepithelial deposit was seen in the aging cohort with no vestibular symptoms, and the thickness of the deposit was greater with advancing age. However, the thickness of the subepithelial deposit in the three cristae of the three subject cases described here was significantly greater than age-related controls. Thus, age alone did not fully explain the thickness of the subepithelial deposit seen in the three subject cases.

Other causes of vestibular symptoms such as vestibular neuritis, and cupulolithiasis were also considered as possibly causative of the thick subepithelial deposit. However, there were no significant differences in the thickness of the deposit between affected and unaffected sides in cases with these disorders, and the thickness of the deposit found in the three subject cases was significantly greater than that found in the control cases with vestibular symptoms.

Because the three reported cases demonstrated focal degeneration of the vestibular neuroepithelium of the cristae, the possible role of degeneration of the neuroepithelium as causative of the thick deposit was considered. Therefore, ears with severe non-focal degeneration were compared with age-matched controls. Although the deposit was thicker than in age-matched controls, the thickness of the deposit of the three subject cases was significantly greater than that in severe non-focal degeneration of the vestibular neuroepithelium.

Because the thickness of the subepithelial deposit found in the three cristae in both ears in the three subject cases was significantly greater than that observed in all control cases, including those with vestibular symptoms or vestibular degeneration, a unique pattern of degeneration seems to exist.

The PAS staining suggests the presence of polysaccharide in the subepithelial deposit. During embryological development of the cupula, mucopolysaccharide is secreted from supporting cells (17) suggesting the possibility that the subepithelial deposit might be released from degenerating supporting cells in the crista.

The finding of deposits of drusen in or near Bruch's membrane, which serves as a basement membrane between retinal pigment epithelia and the choriocapillaris of the retina, are similar to the subepithelial deposit seen in the vestibular neuroepithelium reported here. Both deposits are PAS+ (18). Drusen has been described in advancing age and in macular degeneration (19–24). Drusen is thought to be the cause of, or result of, degeneration of the retinal pigment epithelium. Similarly, the subepithelial deposit in the vestibular subject epithelium may be causally related to the focal degeneration described in these three subject cases.

In summary the average maximum thickness of the subepithelial amorphous deposit in the three cristae of the three subject cases was significantly greater than that of all other cohorts

(aging, Meniere's disease, vestibular neuritis, cupulolithiasis, severe non-focal degeneration of the vestibular neuroepithelium, and Alport syndrome). The thickness of the subepithelial deposit seen in controls in the three cristae was significantly correlated with age. The thickness of the subepithelial deposit seen in controls with severe non-focal degeneration of the neuroepithelium was significantly thicker than age-match controls except for the posterior semicircular canal.

Why the subepithelial deposit is thicker in the three cristae than in the maculae, why the subepithelial deposit is thicker in these three subject cases than in other cases of vestibular neuroepithelial degeneration, and why the deposit is thickest near the summit of the cristae are unknown.

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Fig. 1.
 Cytochleogram of right and left ears of cases 1–3. Missing cytological elements are shown in black.

Fig. 2.
Histopathology of vestibular end organs of subject cases (H&E stain).
Crista of the superior semicircular canal of case 1. A subepithelial deposit (SED) was
thickest near an area of focal degeneration of hair cells (FD) which resulted in thinning of
the neuroepithelium near the summit of the crista (arrow).

Fig. 2B.

Crista of the posterior semicircular canal of case 3. A subepithelial deposit (SED) was seen below an area of focal degeneration of hair cells (FD) resulting in a cellular monolayer near the summit of the crista (arrow).

Fig. 2C.
Macula utriculi of case 1. There was a thin subepithelial deposit (SED) beneath a normal appearing neuroepithelium.

Fig. 3.

Immunostaining, PAS and Gomori trichrome stains.

Anti-myosin VIIa immunostaining of posterior semicircular canal of case 3. The stereocilia and cuticular plate (ST) were immunostained. There was focal degeneration (FD) of hair cells near the summit of the crista.

Fig. 3B.
Anti-myosin VIIa immunostaining of crista of lateral semicircular canal in a control case. Staining of the stereocilia and cuticular plates (ST) of hair cells showed minimal degeneration of hair cells.

Fig. 3C.
Anti-neurofilament immunostaining of crista of the lateral semicircular canal in case 1.
There were reduced numbers of nerve fibers (NF) which traversed the subepithelial mesenchyme and also fibers (PF) that were parallel to the base of the hair cells.

Fig. 3D.

Anti-neurofilament immunostaining of lateral semicircular canal in a control ear. Compared to Fig. 3C, more neurons (NF) traversed the mesenchyme, particularly near the summit of the crista, and also, more nerve fibers (PF) were present parallel to the base of hair cells.

Fig. 3E.

PAS staining of the ampulla of the posterior semicircular canal of case 1. A subepithelial deposit (SED) and the basement membrane (BM) located in the lateral walls of the crista were prominently stained. Focal degeneration (FD) of the neuroepithelium was demonstrated.

Fig. 3F.

Gomori trichrome staining of the crista of the posterior semicircular canal in case 1. The subepithelial deposit (SED) was prominently stained near the summit of the crista. There was focal degeneration (FD) of the hair cells of the neuroepithelium near the summit of the crista.

Fig. 3G.

Anti-collagen 4a1 immunostaining of the crista of the lateral semicircular canal of case 1. The basement membrane (BM) was distinguishable from the subepithelial deposit (SED) which is only lightly stained. The basement membrane was fragmented in the area near the summit of the crista (arrows).

Fig. 4.

Comparison of the Thickness of the Subepithelial Deposit. SSC = superior semicircular canal; LSC= lateral semicircular canal; PSC= posterior semicircular canal; Ut = utricle; Sac = saccule. The asterisks (*) indicates the statistical significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Mann-Whitney U test).

The mean thickness (micrometers) of the subepithelial deposit seen in the three cristae and both maculae of the three study cases verses age-matched controls (n=22; 80 to 99 years old; mean 87.2 years), and Meniere's disease, vestibular neuritis, cupulolithiasis, severe non-

focal vestibular degeneration, and Alport syndrome. The subepithelial deposit was considerably thicker in the three study cases in the three cristae and only slightly thicker in the utricle and saccule.

The mean thickness of the subepithelial deposit of affected ears verses unaffected (Meniere's disease, vestibular neuritis, cupulolithiasis) or age-matched controls (severe non-focal vestibular degeneration, Alport syndrome) in the vestibular end organs. The age-matched controls for the cases of severe non-focal vestibular degeneration and Alport syndrome consisted of 63 ears (range: 42 to 92 years old, mean 68.7 years) and 43 ears (range: 12 to 60, mean 39.1 years), respectively.

Fig. 5.

Scatter plot of aging population without vestibular symptoms (n=111) versus thickness of the subepithelial deposit in vestibular end organs. The subepithelial deposit was consistently thicker in the three study cases (blue dots) in the three cristae and only slightly thicker in the utricle and saccule than in the aging population (red diamonds). Spearman's correlation coefficient by rank test demonstrated moderate correlation with SSC and PSC, and a weak correlation with LSC between age and thickness.

TABLE 1A.

Clinical demographics and average maximum thickness (μm) of the subepithelial deposit and Scarpa's ganglion cell counts of the three subject cases.

Case	Age/ Sex	Vestibular Symptoms	Auditory Findings	Tentative Clinical Diagnosis	Side	SSC (±SD)	(n)	LSC (±SD)	(n)	PSC (±SD)	(n)	Ut (±SD)	(n)	Sac (±SD)	(n)	Total Scarpa's Ganglion Cell
Average Maximum Thickness (µm)																
Count (count/normal *)																
1	89/M	Three one- week episodes of dizziness at ages 26, 35, and 52	Progressive, non-fluctuating bilateral downsloping SNHL	Possible Meniere's disease	R	31.3 ±4.6	(3)	31.4 ±17.0	(6)	29.8 ±12.4	(7)	11.6 ±3.8	(8)	8.7 ±3.4	(9)	N/A
					L	29.5 ±12.8	(5)	27.4 ±10.6	(5)	35.8 ±24.6	(6)	7.8 ±3.3	(6)	7.5 ±1.5	(7)	10,860 (58.6%)
2	89/F	Chronic balance disorder >10 yrs. With 3 episodes of vertigo	Progressive bilateral downsloping SNHL > 40 yrs.	Possible benign positional vertigo, labyrinthitis or vertebrobasilar insufficiency	R	25.4 ±11.0	(4)	20.7 ±11.4	(6)	24.7 ±10.0	(6)	4.8 ±0.9	(3)	7.9 ±3.3	(11)	11,900 (64.2%)
					L	21.2 ±6.4	(3)	24.9 ±14.5	(5)	30.0 ±14.5	(5)	9.2 ±2.6	(4)	8.9 ±2.5	(8)	11,064 (59.7%)
3	82/M	“Dizziness” >10 yrs.	Progressive bilateral downsloping SNHL > 10 yrs.	Unknown	R	26.2 ±10.8	(4)	20.6 ±5.5	(6)	23.0 ±7.6	(7)	9.1 ±1.4	(6)	8.8 ±1.4	(10)	13,192 (71.2%)
					L	20.7 ±4.9	(4)	18.8 ±7.8	(6)	18.8 ±6.6	(7)	8.8 ±1.0	(7)	7.6 ±1.4	(10)	12,430 (67.1%)
mean						23.3 ±6.1		22.7 ±4.7		24.4 ±7.1		7.4 ±2.8		7.8 ±1.0		11,889.2 ±965 (64.1%)

SSC, superior semicircular canal; LSC, lateral semicircular canal; PSC, posterior semicircular canal; Ut, utricle; Sac, saccule. SD, standard deviation; n, number of cross sections. R-right; L-left.

*normal, normal Scarpa's ganglion cell count=18,532 ($\pm$ 1755)

TABLE 1B.Mean thickness (μm) of the subepithelial deposit of normal controls.

Age (years)	(n)	SSC ($\pm$ SD)	LSC ($\pm$ SD)	PSC ($\pm$ SD)	Ut ($\pm$ SD)	Sac ($\pm$ SD)
0	11	9.2 $\pm$ 3.3	9.7 $\pm$ 2.3	9.4 $\pm$ 3.0	6.2 $\pm$ 0.9	5.7 $\pm$ 1.0
1–9	7	9.8 $\pm$ 2.3	11.7 $\pm$ 1.8	12.1 $\pm$ 2.1	6.9 $\pm$ 1.1	5.6 $\pm$ 1.1
10–19	5	9.0 $\pm$ 2.6	8.4 $\pm$ 2.2	8.5 $\pm$ 2.1	5.2 $\pm$ 1.8	5.7 $\pm$ 2.0
20–29	10	8.3 $\pm$ 2.8	9.4 $\pm$ 3.3	9.8 $\pm$ 2.9	5.1 $\pm$ 1.5	4.6 $\pm$ 1.8
30–39	6	11.5 $\pm$ 2.5	12.8 $\pm$ 2.3	11.1 $\pm$ 4.3	6.1 $\pm$ 0.9	4.7 $\pm$ 0.7
40–49	11	10.0 $\pm$ 2.4	9.5 $\pm$ 1.7	12.1 $\pm$ 3.2	5.3 $\pm$ 1.2	4.6 $\pm$ 0.7
50–59	11	11.6 $\pm$ 4.1	12.5 $\pm$ 4.6	12.8 $\pm$ 2.3	6.0 $\pm$ 1.8	5.7 $\pm$ 1.4
60–69	11	11.2 $\pm$ 3.6	11.1 $\pm$ 2.5	12.0 $\pm$ 2.9	5.8 $\pm$ 1.2	4.9 $\pm$ 1.2
70–79	16	12.0 $\pm$ 5.4	13.1 $\pm$ 3.3	14.0 $\pm$ 2.9	6.0 $\pm$ 1.2	5.4 $\pm$ 0.9
80–89	12	14.1 $\pm$ 4.2	13.9 $\pm$ 4.5	13.3 $\pm$ 2.7	6.9 $\pm$ 1.7	6.1 $\pm$ 1.5
90–100	11	15.0 $\pm$ 5.8	14.1 $\pm$ 4.7	17.2 $\pm$ 3.5	6.2 $\pm$ 1.7	5.8 $\pm$ 1.5
Total	111	11.3 $\pm$ 3.8	11.7 $\pm$ 3.7	12.4 $\pm$ 3.6	6.0 $\pm$ 1.5	5.4 $\pm$ 1.3

SSC, superior semicircular canal; LSC, lateral semicircular canal; PSC, posterior semicircular canal; Ut, utricle; Sac, saccule. SD, standard deviation; n, number of ears.