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Temporal Bone Histopathology in *NOG*-Symphalangism Spectrum Disorder

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Abstract

Objective—To describe the human temporal bone histopathology in *NOG*-related Symphalangism Spectrum Disorder, a spectrum of congenital stapes fixation syndromes caused by mutations in the *NOG* gene. To discuss implications for clinical management.

Patient—A patient with a mutation in the *NOG* gene

Intervention(s)—Removal of temporal bones, post-mortem temporal bone computed tomography, histologic processing and review of temporal bones

Main Outcome Measure(s)—Temporal bone histopathology, and correlation with clinical, genetic, audiologic, and radiologic evaluations

Results—Both temporal bones demonstrated fixation of the stapes footplate to the otic capsule due to a circumferential bridge of calcified cartilage. In the right ear (unoperated), there was no additional abnormality of the ossicles or ossicular joints. In the left ear, fenestrations of the stapes footplate and the lateral semicircular canal were seen, consistent with a history of stapedectomy and fenestration procedure. Severe loss of spiral ganglion neurons (SGN) throughout the left cochlea accounted for the profound sensorineural hearing loss; there was a normal number of SGN in the right ear. In both ears, the cochleae demonstrated grossly preserved organs of Corti.

Conclusion—The temporal bone pathologic correlate for conductive hearing loss in this patient with a *NOG* mutation was circumferentially calcified cartilage bridging the stapedo-vestibular joint space. The temporal bone histopathology findings suggest that conductive hearing loss related to *NOG* mutation should be improved after stapedectomy; however, care must be taken in

extrapolating to all patients with *NOG* mutations, as there may be variability in the pathology, especially given the variability of *NOG* spectrum disorders.

Keywords

NOG; symphalangism; conductive hearing loss; stapedectomy

Introduction

Mutations in the *NOG* gene are responsible for multiple rare, autosomal dominant syndromes that include congenital conductive hearing loss and skeletal abnormalities. Noggin, the protein encoded by the *NOG* gene, acts as an antagonist of bone morphogenetic proteins (BMPs) and is critical in normal skeletal and joint development.^{1,2} Three of the *NOG*-Related Symphalangism Spectrum Disorders (*NOG*-SSD) demonstrate hearing loss: (1) proximal symphalangism (SYM1), (2) multiple synostoses syndrome (SYNS1), and (3) stapes ankylosis with broad thumbs and toes (SABTT) (which includes Teunissen-Cremers Syndrome and its variants).^{3, 4} Hearing loss is predominantly conductive in these syndromes, and related to stapes fixation. Evidence for stapes fixation is based on clinical findings of a normal tympanic membrane, conductive hearing loss, absent stapedial reflexes, and operative findings of stapes fixation. Not surprisingly, many of these patients are initially diagnosed with otosclerosis, especially when the onset of hearing loss is unclear and skeletal abnormalities are subtle. Multiple authors have reported improvement of conductive hearing loss after stapedectomy in patients with *NOG* related syndromes.³⁻⁷ Although temporal bone histopathology in patients with congenital stapes ankylosis has been described, the histopathology specific to a confirmed case of one of these rare *NOG*-related syndromes has not yet been reported. We describe here the temporal bone histopathology, clinical course, audiometry, and radiology findings in a patient with a Stapes Ankylosis with Broad Thumbs and Toes Syndrome resulting from a heterozygous nonsense mutation in the *NOG* gene. These histopathology findings have clinical implications for the surgical management of these patients.

Methods

Clinical history, audiologic evaluations, and genetic testing results were collected during life through enrollment in the National Institute on Deafness and Other Communication Disorders (NIDCD) National Temporal Bone Registry.

After death, the temporal bones were fixed in formalin. High resolution computed tomography (CT) imaging was then performed on the temporal bone specimens with the same protocol used clinically. A 40-slice multidetector CT scanner (SOMATOM Sensation; Siemens), with 0.6-mm collimation was used to generate images with resultant 0.5-mm slice thickness.

The temporal bones then underwent standard processing for histologic examination, including decalcification with ethylenediamine tetraacetic acid (EDTA) and celloidin embedding. The specimens were sectioned serially in the horizontal plane at a section

thickness of 20 μm . Every tenth section was stained with Hematoxylin and eosin and mounted on a glass slide.⁸

The slides were examined by light microscopy. Axial CT images were reviewed with a neuro-radiologist blinded to the histological findings.

Results

Case History

This patient reported that he was born with bilateral hearing loss, although this was not diagnosed until approximately 5 years old. His bilateral congenital hearing loss was progressive, and he began wearing hearing aids at age 22. He was diagnosed with otosclerosis and underwent a left fenestration procedure at age 34, and then a left stapedectomy at age 37. He had an initial slight improvement in hearing after the left ear stapedectomy, but subsequently developed a severe to profound mixed hearing loss in that ear. The time course of his left post-operative hearing loss is unknown. He wore a right hearing aid only and was dependent on his right ear for hearing for many years. Clinic notes indicate he had a stable profound hearing loss in the left ear for decades prior to his death. An audiogram done at age 66, 10 years prior to his death, demonstrates right mixed hearing loss, and profound hearing loss on the left (Figure 1). At age 67, he was examined by a geneticist and diagnosed with Stapes Ankylosis with Broad Thumbs and Toes. He underwent skeletal x-rays, which identified short, broad middle and distal phalanges of the thumbs bilaterally, and symphalangism (fusion of the distal and middle phalanges) of the right foot only. He was also noted to have hyperopia, a broad sloping forehead, bulbous nasal tip, and limited cervical range of motion. Genetic testing identified a heterozygous nonsense mutation in the *NOG* gene (p.Q110X).⁹ His family history was significant for conductive hearing loss, which was inherited in an autosomal dominant pattern with complete penetrance. The same mutation was identified in multiple family members, all of whom manifested phenotypic features of SABTT.⁹ Radiologic evaluation of the temporal bones with high resolution CT (Figure 2):

There was no radiographic evidence of otosclerotic foci in either ear.

The right ear demonstrated an intact and normal appearing ossicular chain, with no evidence of malleus or incus fixation.

The left ear demonstrated a canal wall down mastoidectomy cavity with fenestration of the lateral semicircular canal. The incus had been removed, and only a remnant of the malleus remained. A fenestration of the stapes footplate was identified, and the superstructure was missing.

Histopathology

Both ears were notable for stapes fixation due to obliteration of the stapedovestibular joint space with ossified cartilage (Figures 3, 4). These findings account for his conductive hearing loss in life. There were no histologic foci of otosclerosis in either ear. The ossified cartilage at the stapedovestibular joint space had the typical appearance of cartilage with

Notably, high resolution temporal bone CT did not identify the pathologic mineralized cartilage seen by histopathology in the stapedo-vestibular joint space. Since the normal stapedo-vestibular joint space is not always seen as a distinct space on high resolution CT, it may not be possible to confirm these pathologic changes on CT. CT is still useful in the evaluation of patients with suspected *NOG* related hearing loss, however, as CT can identify other etiologies of conductive hearing loss, including malleus fixation, otosclerosis, and third window syndromes.^{15,16}

Multiple authors have reported operative findings of stapes fixation with improvement of conductive hearing loss after stapedectomy in patients with *NOG* related syndromes.³⁻⁷ However, some have reported that stapedectomy is less successful in closing the air-bone gap in these patients compared to patients with otosclerosis. In this case, in the unoperated right ear, there are no temporal bone pathology findings that would suggest an increased likelihood of sensorineural hearing loss or reason for persistent conductive hearing loss. Specifically, there was no malleus fixation or additional ossicular chain abnormalities identified. Thus, based on the temporal bone pathology, a successful result from right stapedectomy would be expected. In the left ear, the stapedectomy was performed after a prior fenestration procedure, and chances for successful hearing result were reduced by the absence of an incus, fibroadipose tissue in the middle ear, and prior fenestration of the labyrinth.

The cause of the post-operative sensorineural hearing loss in the left ear is unknown. There was no significant evidence of a traumatic stapedectomy, such as displaced stapes footplate fragments within the vestibule or injury to the saccular membrane. There was no saccular hydrops that might have pre-disposed the patient to saccular injury. There was no significant evidence of labyrinthitis, such as significant soft tissue fibrosis, new bone formation or lymphocytic infiltrate. The notable pathologic correlate to the profound sensorineural hearing loss in his left ear was the significant loss of spiral ganglion neurons, as compared to an age-matched reference population^{10,11} and compared to his contralateral ear with relatively preserved bone thresholds. Classic experiments in cats have demonstrated that pure tone thresholds remain normal with up to 80% loss of auditory nerve fibers.¹⁷ Thus, while the 69% loss of spiral ganglion neurons in this case was significant, there may be additional mechanisms contributing to his post-operative sensorineural hearing loss. Notably, there was a disproportionate loss of the peripheral dendrites of the spiral ganglion neurons, with relative preservation of the axons, suggesting a retrograde degeneration. There was also minor patchy inner hair cell loss, but not enough to account for the profound hearing loss. In summary, there were no findings at the light microscopic level that appear to have conferred an increased risk of sensorineural hearing loss with stapedectomy in this patient with *NOG* mutation. Generalization of these findings to *NOG*-Related Symphalangism Spectrum Disorder (*NOG*-SSD) patients, however, must be cautioned given this is a single pathologic report and there is known heterogeneity in the genotypes and phenotypes of these patients.

In the right ear, there was no specific pathologic correlate to explain the moderately elevated high frequency bone thresholds. Although there was some loss of hair cells and stria vascularis, the loss is distributed throughout the cochlea. There may be other mechanisms of

sensorineural hearing loss not well seen at the light microscopic level, including degeneration of ribbon synapses and dendrites.

Conclusion

A heterozygous nonsense mutation in *NOG* resulted in Stapes Ankylosis with Broad Thumbs and Toes Syndrome in this patient. The temporal bone pathologic correlate for conductive hearing loss in this patient was circumferentially calcified cartilage crossing the stapedo-vestibular joint space in place of the normal annular ligament. The histopathological findings in this case suggest that conductive hearing loss related to *NOG* mutation should be improved after stapedectomy; however, care must be taken in extrapolating to all *NOG* mutation patients as there may be variability in the pathology.

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Figure 1.

Audio-cytochleogram: Patient's last audiogram, done nine years prior to death at age 69, is shown in the top panel. The recorded word recognition score of 60% on the left is doubtful and probably due to crossover from the right ear due to insufficient masking. Below this, quantification of inner hair cells, outer cells, stria vascularis, and spiral ganglion neuron cell bodies over the length of the cochlea from apex to base is shown. Black areas represent missing elements or % loss.

Figure 2.

Post-mortem high resolution computed tomography performed on the temporal bone specimens. Axial images are shown. Note the normal appearance of the stapes footplate in A, normal incudomalleolar joint in B, stapes footplate fenestration from prior stapedectomy in C, lateral semicircular canal fenestration in D. A,B – right ear. C, D – left ear.

Figure 3. Histological sections through the right (unoperated) temporal bone (Hematoxylin-eosin). A- Low magnification section through stapes footplate, demonstrating fixation at the anterior and posterior margins (1.25X). B- High magnification section demonstrating ankylosis of the stapes footplate to the otic capsule anteriorly (20X).

Figure 4.

Histological sections through the left (operated) temporal bone (Hematoxylin-eosin). A- Low magnification section through stapes footplate, demonstrating fixation at the anterior and posterior margins, as well as evidence of the canal wall down cavity (1.25X). B- High magnification section demonstrating ankylosis of the stapes footplate to the otic capsule anteriorly, essentially the same as the right ear (20X). C – Obliteration of the lateral semicircular canal fenestration with fibrous tissue and new bone growth (4X). D -The organ of Corti and stria vascularis were generally preserved, and there was no cochlear hydrops (10X).