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Temporal Bone Histopathology of X-linked Inherited Alport Syndrome

Omer J. Ungar, MD; Joseph B. Nadol, MD; Felipe Santos, MD

Objective: To describe the histopathologic findings within the human cochlea in X-linked Alport syndrome.
Study Design: Histopathologic analysis of cellular elements within the human cochlea by light microscopy.
Materials and Methods: A right and a left cochleae of a man with genetically confirmed X-linked Alport syndrome was studied post-mortem. The temporal bones underwent standard processing for histologic examination. The slides were examined by light microscopy. Graphic reconstruction of the cochlea was performed to quantify hair cells, pathologic changes of the stria vascularis, and loss of cochlear neuronal cells.
Results: There was severe loss of inner hair cells and all three rows of outer hair cells in the apical two turns of the cochlea. The stria vascularis and spiral ligament showed areas of marked loss which became more prominent from base to apex in each ear. The spiral ganglion cell count in the Rosenthal's canal exhibited loss of 20% to 45% compared to matched historical controls. There was a zone of separation between the organ of Corti and the basilar membrane extending along the basal surface of Deiters cells, Hensen cells, Claudius cells and external sulcus cells. The tunnel of Corti and the space of Nuel were filled with cellular elements along the cochlea.
Conclusion: The histopathologic findings of cochlear involvement in Alport's syndrome are basement membrane separation from the cells of the organ of Corti, outer and inner hair cell loss, and cellular infilling of the tunnel and extracellular spaces of the organ of Corti. These observations contribute to our understanding of the mechanism of sensorineural hearing loss in these patients.
Key Words: Alport's syndrome, temporal bone histopathology, otopathology, temporal bone histology.
Level of Evidence:

INTRODUCTION

Hereditary nephritis (Alport syndrome [AS]) is a clinical syndrome that includes, usually sequentially manifested: progressive glomerular disease, ocular changes and sensorineural hearing loss (SNHL).[1,2] AS is the result of nonsense or missense mutations[3] in at least one of the COL4A5, COL4A3, or COL4A4 genes[4] encoding for collagen type IV α5 chain, α3, and α4 chains, respectively, which form a heterotrimer expressed in the basement membranes (BM) of the glomerulus, cochlea, and eye.[5] The most common mode of inheritance is X-linked[6] (80%), followed by autosomal recessive (15%), and autosomal dominant (5%).

The progressive manifestation of renal pathology in AS is the result of changes in BM composition during development, a process referred to as isotype switching.[7,8] In the unaffected condition, congenitally expressed α1 and α2 chains are replaced by α3, α4, and α5 chains. In affected individuals this process does not occur which is thought to result in a structurally and functionally defective BM accounting for the clinical syndrome.

Diagnosis is made by monoclonal antibody testing against COL4A5 gene extracted from a skin biopsy. Genotype-to-phenotype correlation is usually absent, and large intra-familial phenotypic heterogeneity is common.[9]

We describe here the clinical course and histopathologic findings of both temporal bones of a male patient with a confirmed X-linked AS and review the available otopathologic literature.

MATERIALS AND METHODS

The clinical history was collected during life through enrollment in the National Institute on Deafness and Other Communication Disorders (NIDCD), National Temporal Bone, Hearing, and Balance Pathology Resource Registry. After death, both temporal bones were prepared for light microscopy by fixation in formalin (post-mortem time was 41 hours) followed by standard processing for histologic examination, including decalcification with ethylenediamine tetra-acetic acid (EDTA) and celloidin embedding. Both specimens were sectioned serially in

From the Departments of Otolaryngology Head and Neck Surgery and Maxillofacial Surgery (O.J.U.), Tel-Aviv Sourasky Medical Center, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel; the Department of Otolaryngology (J.B.N., F.S.), Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, U.S.A.; and the the Department of Otolaryngology (J.B.N., F.S.), Harvard Medical School, Boston, Massachusetts, U.S.A.

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Send correspondence to Felipe Santos, MD, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114. Email: felipe_santos@meei.harvard.edu

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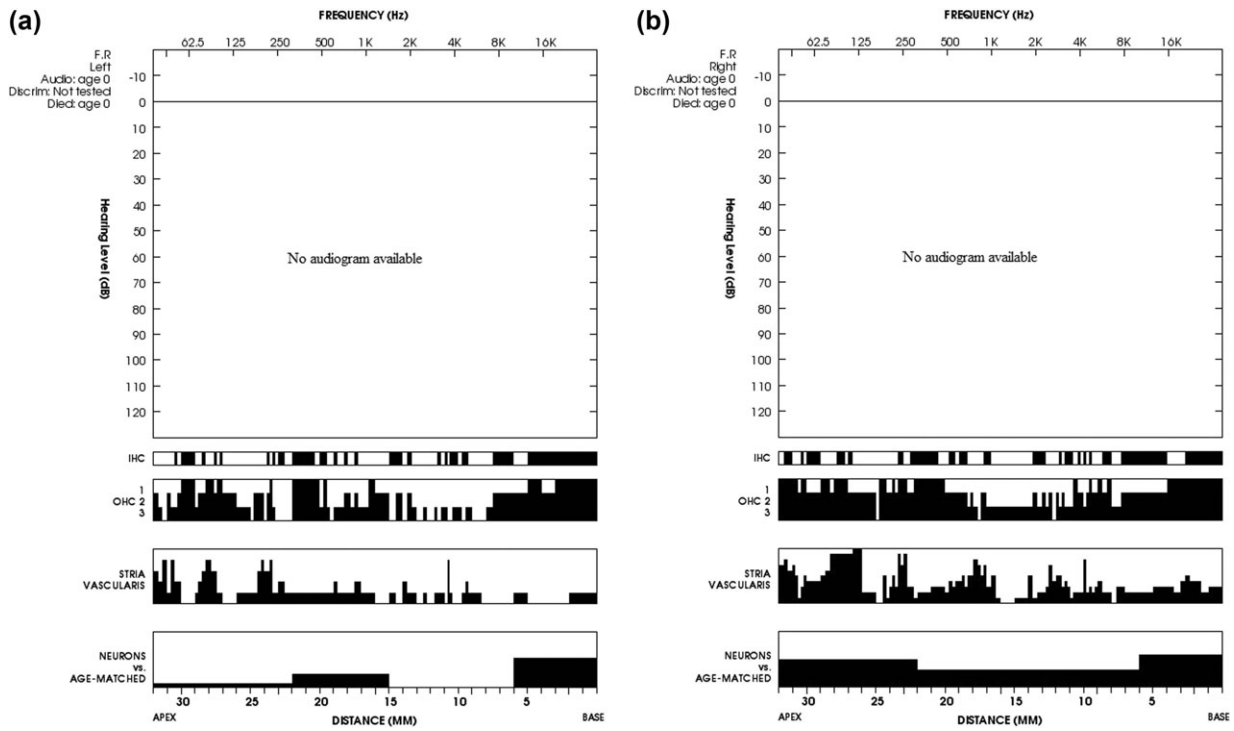

Fig. 1. **a** and **b**: Cytocochleogram of the left (a) and right (b) inner ear of the case presentation. The black areas on the cytocochleogram represent missing or abnormal elements. The inner and outer hair cells are shown as present (white) or absent (black).Vertical axes of the cytocochleogram for the stria vascularis and cochlear neurons represent percentage of loss.

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. The slides were examined by light microscopy.

Two-dimensional graphic reconstruction of the cochlea was performed using published accepted methods[10] to quantify cellular and acellular elements. Hair cells were recorded as being present or absent. Presence of stereocilia (at high magnification of X400–1,000) was the criterion used to determine whether a cell within the organ of Corti was a hair cell or not. Atrophy of the stria vascularis was estimated as a percent of normal in 10% increments. The number of cochlear neuronal cells was counted, calculated and expressed as a percentage of normal for age-matched control subjects.

RESULTS

This male patient was diagnosed with X-linked Alport syndrome in childhood. His mother, daughter and granddaughter carried the trait but not the symptoms of the disease. The familial history was confirmed by genetic testing. At the age of 28 he developed end-stage renal disease and became dependent on hemodialysis. At the age of 33 he underwent a cadaveric renal transplantation that ended up in a graft rejection. Ten years later, he developed SNHL and used hearing aids bilaterally. Audiograms were not available for review. He used eye glasses due to bilateral lenticonus. In his seventh decade, he developed persistent atrial fibrillation, coronary artery disease, and aortic stenosis. He died of myocardial infarction and aspiration pneumonia at the age of 71.

The temporal bones specimens were complete with moderate post-mortem autolysis throughout the specimens. The histopathological findings were similar in both ears. There was a severe loss of inner and all three rows of outer hair cells. This was most pronounced in the apical two turns of the cochlea as graphically represented in the cytocochleogram. The stria vascularis and spiral ligament showed areas of marked loss especially in the base. The spiral ganglion cell count in Rosenthal's canal exhibited 20% and 45% loss (in the left and right cochlea, respectively), compared to matched controls (Figs. 1a and 1b). The tectorial membrane appears normal throughout the cochlea.

Beginning in the upper basal turn there was a zone of separation between the organ of Corti and the basilar membrane extending along the basilar surface of the Deiter cells, Hensen cells, Claudius cells, and external sulcus cells (Fig. 2) along the cochlear duct (Fig. 3). The tunnel of Corti and space of Nuel were filled with cellular elements along the cochlea (Fig. 4).

DISCUSSION

AS results from mutations in genes encoding for α3, α4 and α5 chains of type IV collagen. These mutations result in renal pathology as seen by light microscopy of an affected individual.[8] There is a thickening of the glomerular basement membrane (GMB) and splitting of the lamina densa. The defect in the GBM in AS results in hematuria during childhood and proteinuria develops during the second or third decades with renal failure as the final outcome[8,11] as in the case presented in this study.

In the AS-affected human cochlea, separation of the organ of Corti from the basilar membrane is a common observation in human temporal bones. In the largest

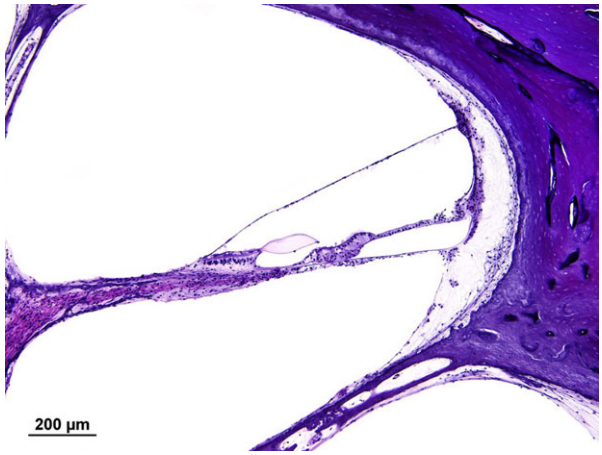

Fig. 2. Right middle cochlear duct turn. Horizontal section, light microscopy, Hematoxylin, and Eosin staining. A zone of separation between the organ of Corti and the basilar membrane extending along the bottom surface of the Deiter cells, Hensen cells, Claudius cells, and external sulcus cells is clearly seen.

case series published, separation of the organ of Corti from the underlying basilar membrane was observed in eight of nine cases.[12] The same observation was reported in animal models.[13] The absence of this finding[14,15] likely reflects phenotypic heterogeneity.[9] Separation of the organ of Corti from the basilar membrane is unique to AS and is not seen in normal or otherwise pathologic histological specimens.[10] This separation is thought to be the result of a structurally defective BM that fails to provide adequate adhesive support between the basilar membrane and the organ of Corti. Given the fact that α3, α4, and probably α5 chains of type IV collagen are found in the BM under the organ of Corti,[16] this separation may affect cochlear micromechanics and be the pathological mechanism that leads to SNHL in affected individuals. Affected individuals with AS show sensorineural loss at frequencies that correspond to the areas of BM separation in our histological specimens. Speech

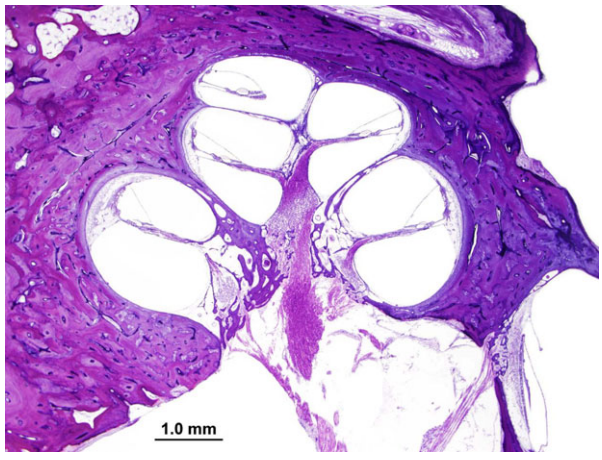

Fig. 3. Mid-modiolar section of the right cochlea. Light microscopy, Hematoxylin, and Eosin staining. The zone of separation which is magnified in Figure 2, is clearly seen to present along the whole cochlear duct.

Fig. 4. Right middle cochlear duct turn. Horizontal section, light microscopy, Hematoxylin, and Eosin staining. The tunnel of Corti (arrow) and space of Nuel (arrow head) are filled with cellular elements along the cochlea.

discrimination usually remains excellent in AS, and pure tone audiometry is rarely worse than 60 to 70 dB.[10,17]

One must consider the possibility that the observed separation in the cochlea is a post-mortem artifact. The basilar membrane may be structurally vulnerable to sheering and separation during fixation and histological processing, ie, the zonal separation may be a post-mortem artifact of a pre-mortem pathology. Arguing against this possibility is that previous descriptions of AS cochlear pathology with post-mortem times as short as 4 hours showed similar findings of separation of the organ of Corti from the basilar membrane,[12] while other temporal bone descriptions with longer post-mortem time did not report BM separation.[14,15] Hence, we can conclude that basilar membrane separation from the organ of Corti is a result of pre-mortem pathology and not of post-mortem artifact.

The second observation is the presence of cells within the extracellular spaces of Nuel and the canal of Corti. Similar findings are seen in normal fetal cochlea prior to tunnel of Corti and Nuel space development. Cellular resorption of these cytological elements typically occurs at 16 to 19 weeks of gestation.[18] It is well known that basement membranes has diverse biologic functions including tissue remodeling and tissue architecture maintenance during in embryonic development.[8,17,19] It is possible that the COL4 mutated basement membrane in AS individuals negatively influences normal development of the adult form of organ of Corti resulting in a fetal type organ of Corti throughout extra-uterine life.

CONCLUSION

The histopathologic cochlear alteration in AS affected individuals includes separation of the Organ of Corti from the basilar membranes, inner and outer hair cell loss, and cellular filling of the tunnel of Corti and

the space of Nuel. The sensorineural hearing loss may be the result of altered cochlear micromechanics.

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