Temporal Bone Histopathology of X-linked Inherited Alport Syndrome

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**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 \(\alpha\) 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 \(\alpha 1\) and \(\alpha 2\) chains are replaced by \(\alpha 3\), \(\alpha 4\), and \(\alpha 5\) 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%).

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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 cellloidin embedding. Both specimens were sectioned serially in

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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 methods10 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. Audio-grams 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 (GBM) 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 outcome8,11 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
case series published, separation of the organ of Corti from the underlying basilar membrane was observed in eight of nine cases. The same observation was reported in animal models. The absence of this finding likely reflects phenotypic heterogeneity. 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. 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, 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 discrimination usually remains excellent in AS, and pure tone audiometry is rarely worse than 60 to 70 dB.

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, i.e., 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, while other temporal bone descriptions with longer post-mortem time did not report BM separation. 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. It is well known that basement membranes have diverse biologic functions including tissue remodeling and tissue architecture maintenance during in embryonic development. 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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BIBLIOGRAPHY
1. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. J Am Soc Nephrol 2013;24:364–375.
2. Alport AC. Hereditary familial congenital haemorrhagic nephritis. Br Med J 1927;1:3454:504.
3. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome natural history in 195 families and genotype-phenotype correlations in males. J Am Soc Nephrol 2000;11:649–657.
4. Hostikka SL, Eddy BL, Byers MG, Hoyby M, Shows TB, Tryggvason K. Identification of a distinct type IV collagen alpha chain with restricted kidney distribution and assignment of its gene to the locus of X chromosome-linked Alport syndrome. Proc Natl Acad Sci U S A 1990;87:1606–1610.
5. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport’s syndrome, Goodpasture’s syndrome, and type IV collagen. N Engl J Med 2003;348:2543–2556.
6. Flinter F, Chantler C, Cameron JS, Houston I, Bohrm M. Genetics of classic Alport’s syndrome. Lancet 1988;332:1005–1007.
7. Miner JH, Sanes JR. Collagen IV alpha 3, alpha 4, and alpha 5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. J Cell Biol 1994;127:879–891.
8. Kashtan CE. Alport syndrome. An inherited disorder of renal, ocular, and cochlear basement membranes. Medicine 1999;78:338–360.
9. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a “European Community Alport Syndrome Concerted Action” study. J Am Soc Nephrol 2002;14:2605–2610.
10. Schuknecht HF. Pathology of the Ear, 2nd ed. New York: Lea and Febiger, 1993.
11. Kashtan CE, Atkin UL, Gregory MC, Michael AF. Identification of variant Alport phenotypes using an Alport-specific antibody probe. Kidney Int 1989;36:669–674.
12. Merchant SN, Burgess BJ, Adams JC, et al. Temporal bone histopathology in Alport syndrome. Laryngoscope 2004;114:1609–1618.
13. Gratton MA, Rao VH, Meehan DT, Askew C, Cosgrove D. Matrix metalloproteinase dysregulation in the stria vascularis of mice with alport syndrome. Am J Pathol 2005;166:1465–1474.
14. Fujita S, Hayden RC. Alport’s syndrome. Arch Otolaryngol 1969;90:75–88.
15. Johnson LG, Arenberg I. Cochlear abnormalities in Alport’s syndrome. Arch Otolaryngol 1981;107:340–349.
16. Kleppel MM, Santi PA, Cameron JD, Wieslander J, Michael AF. Human tissue distribution of novel basement membrane collagen. Am J Pathol 1989;134:813.
17. Gleeson MJ. Alport’s syndrome: audiological manifestations and implications. J Laryngol Otol 1984;98:449–465.
18. Gulya AJ, Schuknecht HF. Anatomy of the Temporal Bone with Surgical Implications, 2nd ed. New York: Parthenon, 1996.
19. Cosgrove D, Samuelson G, Meehan DT, et al. Ultrastructural, physiological, and molecular defects in the inner ear of a gene-knockout mouse model for autosomal Alport syndrome. Hear Res 1998;121:84–98.