Table Ⅳ. Clinical features and genetic analysis of the 5 subjects with BOR/BO

Age/ Gender	Onset of HL	Type of HL (Right / Left)	Severity of HL (Right / Left)	Other findings	Gene / Genotype
23/F	5	SNHL / SNHL	Mild / Moderate	Preauricular pit, EVA	*SIX1* / [Y129C]
21/F	Childhood	SNHL / SNHL	Moderate / Moderate	Preauricular pit, EVA	*SIX1* / [Y129C]
29/F	Childhood	SMHL / MHL	Mild / Moderate	Preauricular pit	*EYA1* / [S189G]
9/M	5	SNHL / MHL	Mild / Moderate	Preauricular pit, Branchial fistula	*EYA1* / [R407Q]
22/F	Childhood	MHL / SHNL	Moderate / Moderate	Preauricular pit, Branchial fistula, Renal anomaly, Pinnae deformity	*EYA1* / [IVS11-1G>A]

diagnosed as nonsyndromic HL.

7. Conclusions

Based on epidemiological data, at least one child in 1000 is born with HL in developed countries and more than 50% of prelingual deafness cases are found to have hereditary HL. Therefore, a good understanding of the deafness genes is important to select the optimal treatment of patients with HL. This article reviewed the clinical and genetic characteristics of the most prevalent deafness genes such as *GJB2*, *SLC26A4*, and the mitochondrial DNA 1555A>G in Japanese. Furthermore, this study also ellucidated the deafness gene, *WFS1*, whose mutation is closely related to low-frequency HL and *SLC26A4*, *EYA1* and *SIX1* which are regarded to cause EVA.

References

1. Smith R, Bale JJ, White K. Sensorineural hearing loss in children. Lancet 2005;365:879-90.
2. Hilgert N, Smith R, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? Mutat Res 2009;681:189-96.
3. Fortnum H, Summerfield A, Marshall D, et al. Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: questionnaire based ascertainment study. BMJ 2001;323:536-40.
4. Morton C, Nance W. Newborn hearing screening--a silent revolution. N Engl J Med 2006;354:2151-64.
5. Wrightson A. Universal newborn hearing screening. Am Fam Physician 2007;75:1349-52.
6. Fukushima K, Mimaki N, Fukuda S, et al. Pilot study of universal newborn hearing screening in Japan: district-based screening program in Okayama. Ann Otol Rhinol Laryngol 2008;117:166-71.
7. Joint Committee on Infant Hearing, American Academy of Audiology, American Academy of Pediatrics, et al. Year 2000 position statement: principles and guidelines for early hearing detection and intervention programs. Joint Committee on Infant Hearing, American Academy of Audiology, American Academy of Pediatrics, American Speech-Language-Hearing Association, and Directors of Speech and Hearing Programs in State Health and Welfare Agencies. Pediatrics 2000;106:798-817.
8. Morton N. Genetic epidemiology of hearing impairment. Ann N Y Acad Sci 1991;630:16-31.
9. Van Camp G, Willems P, Smith R. Nonsyndromic hearing impairment: unparalleled heterogeneity. Am J Hum Genet 1997;60:758-64.
10. Cremers CWRJ, Smith RJ. Genetic hearing impairment. Switzerland: Kager; 2002.
11. Gorlin RJ, Toriello HV, Cohen MM,. Hereditary Hearing Loss and its Syndromes. NewYork: Oxford University Press; 1995.
12. Fraser G. The causes of profound deafness in childhood. Baltimore: Johns Hopkins University Press; 1976.
13. Usami S, Wagatsuma M, Fukuoka H, et al. The responsible genes in Japanese deafness patients and clinical application using Invader assay. Acta Otolaryngol 2008;128:446-54.
14. Fuse Y, Doi K, Hasegawa T, et al. Three novel connexin26 gene mutations in autosomal recessive non-syndromic deafness. Neuroreport 1999;10:1853-7.
15. Kitamura K, Takahashi K, Noguchi Y, et al. Mutations of the Pendred syndrome gene (PDS) in patients with large vestibular aqueduct. Acta Otolaryngol 2000;120:137-41.
16. Noguchi Y, Yashima T, Ito T, et al. Audiovestibular findings in patients with mitochondrial A1555G mutation. Laryngoscope 2004;114:344-8.
17. Kitamura K, Takahashi K, Tamagawa Y, et al. Deafness genes. J Med Dent Sci 2000;47:1-11.
18. Zelante L, Gasparini P, Estivill X, et al. Connexin26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. Hum Mol Genet 1997;

6:1605-9.
19. Estivill X, Fortina P, Surrey S, et al. Connexin-26 mutations in sporadic and inherited sensorineural deafness. Lancet 1998;351:394-8.
20. Kelley P, Harris D, Comer B, et al. Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet 1998;62:792-9.
21. Bruzzone R, White T, Paul D. Connections with connexins: the molecular basis of direct intercellular signaling. Eur J Biochem 1996;238:1-27.
22. Martínez A, Acuña R, Figueroa V, et al. Gap-junction channels dysfunction in deafness and hearing loss. Antioxid Redox Signal 2009;11:309-22.
23. Stauffer K. The gap junction proteins beta 1-connexin (connexin-32) and beta 2-connexin (connexin-26) can form heteromeric hemichannels. J Biol Chem 1995;270:6768-72.
24. Kikuchi T, Kimura R, Paul D, et al. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. Anat Embryol (Berl) 1995;191:101-18.
25. Kelsell D, Dunlop J, Stevens H, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature 1997;387:80-3.
26. Denoyelle F, Lina-Granade G, Plauchu H, et al. Connexin 26 gene linked to a dominant deafness. Nature 1998;393:319-20.
27. Snoeckx R, Huygen P, Feldmann D, et al. GJB2 mutations and degree of hearing loss: a multicenter study. Am J Hum Genet 2005;77:945-57.
28. Green G, Scott D, McDonald J, et al. Carrier rates in the midwestern United States for GJB2 mutations causing inherited deafness. JAMA 1999;281:2211-6.
29. Shahin H, Walsh T, Sobe T, et al. Genetics of congenital deafness in the Palestinian population: multiple connexin 26 alleles with shared origins in the Middle East. Hum Genet 2002;110:284-9.
30. Liu X, Xia X, Ke X, et al. The prevalence of connexin 26 (GJB2) mutations in the Chinese population. Hum Genet 2002;111:394-7.
31. Mahdieh N, Nishimura C, Ali-Madadi K, et al. The frequency of GJB2 mutations and the Delta (GJB6-D13S1830) deletion as a cause of autosomal recessive non-syndromic deafness in the Kurdish population. Clin Genet 2004;65:506-8.
32. Posukh O, Pallares-Ruiz N, Tadinova V, et al. First molecular screening of deafness in the Altai Republic population. BMC Med Genet 2005;6:12.
33. Ohtsuka A, Yuge I, Kimura S, et al. GJB2 deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet 2003;112:329-33.
34. Oguchi T, Ohtsuka A, Hashimoto S, et al. Clinical features of patients with GJB2 (connexin 26) mutations: severity of hearing loss is correlated with genotypes and protein expression patterns. J Hum Genet 2005;50:76-83.
35. Scrimshaw B, Faed J, Tate W, et al. Rapid identification of an A1555G mutation in human mitochondrial DNA implicated in aminoglycoside-induced ototoxicity. J Hum Genet 1999;44:388-90.
36. Kupka S, Tóth T, Wróbel M, et al. Mutation A1555G in the 12S rRNA gene and its epidemiological importance in German, Hungarian, and Polish patients. Hum Mutat 2002;19:308-9.
37. Tekin M, Duman T, Boğoçlu G, et al. Frequency of mtDNA A1555G and A7445G mutations among children with prelingual deafness in Turkey. Eur J Pediatr 2003; 162:154-8.
38. ØStergaard E, Montserrat-Sentis B, Grønskov K, et al. The A1555G mtDNA mutation in Danish hearing-impaired patients: frequency and clinical signs. Clin Genet 2002;62:303-5.
39. Bitner-Glindzicz M, Pembrey M, Duncan A, et al. Prevalence of mitochondrial 1555A>G mutation in European children. N Engl J Med 2009;360:640-2.
40. Vandebona H, Mitchell P, Manwaring N, et al. Prevalence of mitochondrial 1555A>G mutation in adults of European descent. N Engl J Med 2009;360:642-4.
41. Malik S, Pieter N, Sudoyo H, et al. Prevalence of the mitochondrial DNA A1555G mutation in sensorineural deafness patients in island Southeast Asia. J Hum Genet 2003;48:480-3.
42. Prezant T, Agapian J, Bohlman M, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 1993;4:289-94.
43. Fischel-Ghodsian N. Genetic factors in aminoglycoside toxicity. Ann N Y Acad Sci 1999;884:99-109.
44. Guan M, Fischel-Ghodsian N, Attardi G. A biochemical basis for the inherited susceptibility to aminoglycoside ototoxicity. Hum Mol Genet 2000;9:1787-93.
45. Bates D. Aminoglycoside ototoxicity. Drugs Today (Barc) 2003;39:277-85.
46. Estivill X, Govea N, Barceló E, et al. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment of aminoglycosides. Am J Hum Genet 1998;62:27-35.
47. Bykhovskaya Y, Yang H, Taylor K, et al. Modifier locus for mitochondrial DNA disease: linkage and linkage disequilibrium mapping of a nuclear modifier gene for maternally inherited deafness. Genet Med;3:177-80.
48. Johnson K, Zheng Q, Bykhovskaya Y, et al. A nuclear-mitochondrial DNA interaction affecting hearing impairment in mice. Nat Genet 2001;27:191-4.
49. Hyde G, Rubel E. Mitochondrial role in hair cell survival after injury. Otolaryngol Head Neck Surg 1995;113:530-40.
50. del Castillo F, Rodríguez-Ballesteros M, Martín Y, et al. Heteroplasmy for the 1555A>G mutation in the mitochondrial 12S rRNA gene in six Spanish families with non-syndromic hearing loss. J Med Genet 2003;40:632-6.
51. Kawashima Y, Noguchi Y, Ito T, et al. Vestibular evoked myogenic potentials in patients with the mitochondrial A1555G mutation. Laryngoscope 2009;119:1874-9.
52. Lynch E, Lee M, Morrow J, et al. Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of

the Drosophila gene diaphanous. Science 1997; 278: 1315-8.
53. Bespalova I, Van Camp G, Bom S, et al. Mutations in the Wolfram syndrome 1 gene (WFS1) are a common cause of low frequency sensorineural hearing loss. Hum Mol Genet 2001;10:2501-8.
54. Verhoeven K, Van Laer L, Kirschhofer K, et al. Mutations in the human alpha-tectorin gene cause autosomal dominant non-syndromic hearing impairment. Nat Genet 1998;19:60-2.
55. De Leenheer E, Kunst H, McGuirt W, et al. Autosomal dominant inherited hearing impairment caused by a missense mutation in COL11A2 (DFNA13). Arch Otolaryngol Head Neck Surg 2001;127:13-7.
56. Iwasaki S, Harada D, Usami S, et al. Association of clinical features with mutation of TECTA in a family with autosomal dominant hearing loss. Arch Otolaryngol Head Neck Surg 2002;128:913-7.
57. Takeda K, Inoue H, Tanizawa Y, et al. WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. Hum Mol Genet 2001;10:477-84.
58. Cryns K, Sivakumaran T, Van den Ouweland J, et al. Mutational spectrum of the WFS1 gene in Wolfram syndrome, nonsyndromic hearing impairment, diabetes mellitus, and psychiatric disease. Hum Mutat 2003;22:275-87.
59. Hofmann S, Philbrook C, Gerbitz K, et al. Wolfram syndrome: structural and functional analyses of mutant and wild-type wolframin, the WFS1 gene product. Hum Mol Genet 2003;12:2003-12.
60. Cryns K, Thys S, Van Laer L, et al. The WFS1 gene, responsible for low frequency sensorineural hearing loss and Wolfram syndrome, is expressed in a variety of inner ear cells. Histochem Cell Biol 2003;119:247-56.
61. Cryns K, Pfister M, Pennings R, et al. Mutations in the WFS1 gene that cause low-frequency sensorineural hearing loss are small non-inactivating mutations. Hum Genet 2002;110:389-94.
62. Noguchi Y, Yashima T, Hatanaka A, et al. A mutation in Wolfram syndrome type 1 gene in a Japanese family with autosomal dominant low-frequency sensorineural hearing loss. Acta Otolaryngol 2005;125:1189-94.
63. Fukuoka H, Kanda Y, Ohta S, et al. Mutations in the WFS1 gene are a frequent cause of autosomal dominant nonsyndromic low-frequency hearing loss in Japanese. J Hum Genet 2007;52:510-5.
64. Fujikawa T, Noguchi Y, Ito T, et al. Additional heterozygous 2507A>C mutation of WFS1 in progressive hearing loss at lower frequencies. Laryngoscope 2010;120:166-71.
65. Valvassori G, Clemis J. The large vestibular aqueduct syndrome. Laryngoscope 1978;88:723-8.
66. Ito T, Noguchi Y, Yashima T, et al. SIX1 mutation associated with enlargement of the vestibular aqueduct in a patient with branchio-oto syndrome. Laryngoscope 2006;116:796-9.
67. Pendred V. Deaf-mutism and goitre. Lancet II 1896:532.
68. Li X, Everett L, Lalwani A, et al. A mutation in PDS causes non-syndromic recessive deafness. Nat Genet 1998;18:215-7.
69. Everett L, Glaser B, Beck J, et al. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet 1997;17:411-22.
70. Wilcox E, Everett L, Li X, et al. The PDS gene, Pendred syndrome and non-syndromic deafness DFNB4. Adv Otorhinolaryngol 2000;56:145-51.
71. Campbell C, Cucci R, Prasad S, et al. Pendred syndrome, DFNB4, and PDS/SLC26A4 identification of eight novel mutations and possible genotype-phenotype correlations. Hum Mutat 2001;17:403-11.
72. Fugazzola L, Cerutti N, Mannavola D, et al. The role of pendrin in iodide regulation. Exp Clin Endocrinol Diabetes 2001;109:18-22.
73. Royaux I, Suzuki K, Mori A, et al. Pendrin, the protein encoded by the Pendred syndrome gene (PDS), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. Endocrinology 2000;141:839-45.
74. Scott D, Wang R, Kreman T, et al. The Pendred syndrome gene encodes a chloride-iodide transport protein. Nat Genet 1999;21:440-3.
75. Wangemann P, Nakaya K, Wu T, et al. Loss of cochlear HCO3- secretion causes deafness via endolymphatic acidification and inhibition of Ca2+ reabsorption in a Pendred syndrome mouse model. Am J Physiol Renal Physiol 2007;292:F1345-53.
76. Park H, Shaukat S, Liu X, et al. Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness. J Med Genet 2003;40:242-8.
77. Tsukamoto K, Suzuki H, Harada D, et al. Distribution and frequencies of PDS (SLC26A4) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. Eur J Hum Genet 2003;11:916-22.
78. Coyle B, Reardon W, Herbrick J, et al. Molecular analysis of the PDS gene in Pendred syndrome. Hum Mol Genet 1998;7:1105-12.
79. Wang Q, Zhao Y, Rao S, et al. A distinct spectrum of SLC26A4 mutations in patients with enlarged vestibular aqueduct in China. Clin Genet 2007;72:245-54.
80. Park H, Lee S, Jin H, et al. Genetic basis of hearing loss associated with enlarged vestibular aqueducts in Koreans. Clin Genet 2005;67:160-5.
81. Suzuki H, Oshima A, Tsukamoto K, et al. Clinical characteristics and genotype-phenotype correlation of hearing loss patients with SLC26A4 mutations. Acta Otolaryngol 2007;127:1292-7.
82. Smith R, Schwartz C. Branchio-oto-renal syndrome. J Commun Disord 1998;31:411-20; quiz 21.
83. Stinckens C, Standaert L, Casselman J, et al. The presence of a widened vestibular aqueduct and progressive sensorineural hearing loss in the branchio-

oto-renal syndrome. A family study. Int J Pediatr Otorhinolaryngol 2001;59:163-72.
84. Kemperman M, Koch S, Kumar S, et al. Evidence of progression and fluctuation of hearing impairment in branchio-oto-renal syndrome. Int J Audiol 2004;43:523-32.
85. Fraser F, Ling D, Clogg D, et al. Genetic aspects of the BOR syndrome--branchial fistulas, ear pits, hearing loss, and renal anomalies. Am J Med Genet 1978;2:241-52.
86. Ozaki H, Nakamura K, Funahashi J, et al. Six1 controls patterning of the mouse otic vesicle. Development 2004;131:551-62.
87. Zimmerman J, Bui Q, Steingrímsson E, et al. Cloning and characterization of two vertebrate homologs of the Drosophila eyes absent gene. Genome Res 1997;7:128-41.
88. Abdelhak S, Kalatzis V, Heilig R, et al. A human homologue of the Drosophila eyes absent gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. Nat Genet 1997;15:157-64.
89. Xu P, Adams J, Peters H, et al. Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. Nat Genet 1999;23:113-7.
90. Hoskins B, Cramer C, Silvius D, et al. Transcription factor SIX5 is mutated in patients with branchio-oto-renal syndrome. Am J Hum Genet 2007;80:800-4.
91. Chang E, Menezes M, Meyer N, et al. Branchio-oto-renal syndrome: the mutation spectrum in EYA1 and its phenotypic consequences. Hum Mutat 2004;23:582-9.
92. Ruf R, Xu P, Silvius D, et al. SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. Proc Natl Acad Sci U S A 2004;101:8090-5.
93. Yashima T, Noguchi Y, Ishikawa K, et al. Mutation of the EYA1 gene in patients with branchio-oto syndrome. Acta Otolaryngol 2003;123:279-82.

Supplemental Data

In the present review paper, we used our genetic analysis data from 382 unrelated Japanese patients (235 females and 147 males; age, 0-84 y; mean age ± SD, 40.22 ± 18.83 years). The patients visited the Department of Otolaryngology, Tokyo Medical and Dental University between October 1999 and July 2008 and were suspected to have hereditary HL. DNA was extracted from peripheral blood lymphocytes using standard methods after obtaining written informed consent from each donor and/or guardians of the HL children. We first screened for *GJB2* mutation and the mitochondrial DNA 1555A>G for all the 382 patients. The presence of *WFS1* mutations were analyzed for four patients with autosomal dominant low-frequency SNHL. The coding exons including exon-intron boundaries of *GJB2*, *WFS1*, *SLC26A4*, *EYA1*, and *SIX1*; and mitochondrial DNA around the 1555 were amplified by polymerase chain reaction (PCR) in a thermal cycler (model 9700, PE Applied Biosystems, Foster City, CA) as described previously[16,62,66,93]. The mitochondrial DNA 1555A<G was first screened on PCR restriction fragment length polymorphism using *Bsm*A I (New England Biolabs, Beverly, MA). Whenever the 1555A>G mutation was detected, the PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN), and were directly sequenced using the Applied Biosystems Prism BigDye Terminator Cycle Sequencing Ready Kit and an ABI Prism model 310 Genetic Analyzer. The other genetic analysis for *GJB2*, *WFS1*, *SLC26A4*, *EYA1*, and *SIX1* was carried out by direct sequencing. All procedures were approved by the institutional review board at Tokyo Medical and Dental University.

ELSEVIER

Auris Nasus Larynx 37 (2010) 422–426

AURIS NASUS LARYNX
INTERNATIONAL JOURNAL OF ORL & HNS

www.elsevier.com/locate/anl

What do patients with hereditary deafness think of genetic studies?

Shino Abe, Yoshihiro Noguchi *, Ken Kitamura

Department of Otolaryngology, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

Received 1 May 2009; accepted 24 December 2009
Available online 21 February 2010

Abstract

Objective: We conducted an attitude survey for patients with hearing loss (HL). The aim of this study was to investigate the opinions of patients or parents of deaf children regarding the deafness gene, genetic testing and a gene related HL.
Method: A questionnaire was sent to 201 individuals who visited the Department of Otolaryngology, Tokyo Medical and Dental University and who received genetic testing for HL from September 2000 to January 2006. There were 14 questions in the questionnaire that were classified into four topics related to a deafness gene and hereditary HL, genetic testing, outpatient department of medical genetics/genetic counseling, and the results of genetic testing. The study consisted of 140 respondents (70%) of 201 administered surveys.
Result: Before visiting our department, only 36% of the respondents were aware that a genetic factor was a cause of HL. Despite our explanation of a deafness gene and hereditary HL, 23% of 134 respondents answered that they had not received any such explanation. Furthermore, 14% of the 103 respondents who had answered that they receive the explanation, however, they did not fully understand it. Thirty-nine percent of the respondents made their own decision regarding the genetic testing, whereas 53.5% received the tests upon the advice of a physician or family member. In contrast, 91% of the respondents had a positive attitude towards other future genetic tests. The existence of the genetic outpatient department or genetic counseling has been seldom acknowledged, but upon learning of its availability, nearly one third of the respondents indicated that they would like to receive genetic counseling. Although no respondent had social and/or family problems after being informed that they had a deafness gene mutation, some respondents worried about the result.
Conclusion: The results of the survey suggested that the vast majority of the respondents were satisfied with genetic testing for HL and that the barriers to take the genetic test were less than expected. However, some respondents have a negative attitude towards genetic testing and counseling. Furthermore, the issue of disclosure may be burdensome to patients.

Keywords: Attitude survey; Deafness gene; Genetic testing; Genetic counseling

1. Introduction

Hearing loss (HL) due to genetic mutations occurs in a highly frequent clinical state among birth defects [1]. A lot of genes responsible for HL have been identified (Hereditary Hearing Loss Homepage, http://webh01.ua.ac.be/hhh/) and genetic testing is going to be included in the diagnostic protocol of HL. Furthermore, the detection of causative genes may be indispensable in the medical examinations of patients with congenital deafness and/or familial HL in the future. What we should consider is the ethical issues of genetic testing and a correct understanding of the concept of the "gene". In this study, we conducted an attitude survey of the patients who received the genetic testing for HL at the Department of Otolaryngology, Tokyo Medical and Dental University. In this study we inquired about the patients' understanding and their opinions regarding a deafness gene, genetic testing and the gene related to their own HL.

2. Materials and methods

All the procedures were approved by our Institutional Review Board (IRB No. 68) and were carried out only after obtaining the written informed consent from each individual and/or guardians of the children. Less than 10% of the patients refused our proposal to perform genetic testing. Consequently,

* Corresponding author. Tel.: +81 3 5803 5308; fax: +81 3 3813 2134.
E-mail address: noguchi.oto@tmd.ac.jp (Y. Noguchi).

0385-8146/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.anl.2009.12.007

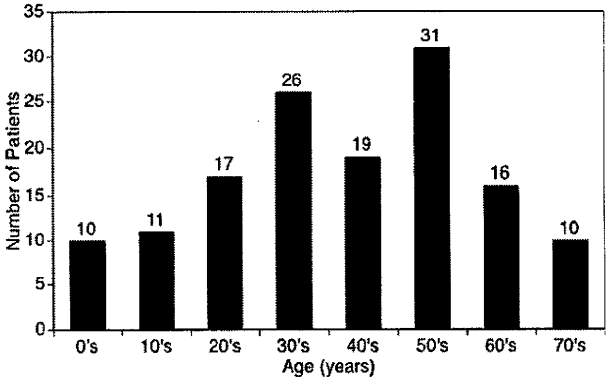

Fig. 1. The age distribution of the 140 patients.

we enrolled 201 individuals who visited the Department of Otolaryngology, Tokyo Medical and Dental University from September 2000 to January 2006 because of HL and all of them agreed to our proposal. These individuals underwent genetic testing and requested the results from the tests. We administered a questionnaire to the 201 individuals and made a study based on the collected responses. The questionnaire comprised 14 questions that were classified into four topics regarding a deafness gene and hereditary HL, genetic testing, outpatient department of medical genetics/genetic counseling, and the results of genetic testing. We asked each individual to fill in the questionnaire that had several arranged answers. When a patient was under the age of 19 years, we asked the guardian of the patient to respond to the survey.

A total of 140 (70%) of the 201 individuals responded to the survey, and they all were fully registered for into the study. Twenty-one (15%) of the 140 respondents were from the guardian of the patient. The patients consisted of 60 males and 80 females. The average age of the 140 patients was 41.6 years and the age ranged from 4 to 78 years (Fig. 1). The patients included 20 with congenital deafness with unknown etiology, 64 with postlingual familiar HL, 9 with syndromic hereditary HL, and 47 with idiopathic bilateral sensorineural HL. Sixteen (11%) of the 140 patients were identified as having any mutations for a deafness gene including *GJB2*, *SLC26A4*, *SIX1*, *ATP6V1B1*, mitochondrial DNA (mtDNA) 12S rRNA gene (1555A>G) and mtDNA $tRNA^{Leu\ (UUR)}$ gene (3243A>G).

The significance of the differences in the yes-or-no answers to question numbers 1, 3, and 9 was calculated by the chi-square test using the Stat View version 4.51 software program (Abacus Concepts, Berkeley, CA, U.S.A.) running on a Macintosh computer.

3. Results

3.1. Deafness gene and hereditary hearing loss

The questions in the questionnaire and the answers from respondents are summarized in Table 1. The total number of

Table 1
Summary of results.

Questions and arranged answers	Proportion (%) (p value)
1. Before receiving the genetic testing did you know that a genetic factor is one of the causes for hearing loss?	
Yes	50/138 (36%)
No	88/138 (64%)
	($p = 0.021$)
2. Those who answered "Yes" to the above question: How did you learn about it? Check all that apply.	
At another hospital	16 (32%)
From friend	7 (14%)
From magazine	9 (18%)
From internet	6 (12%)
Others	14 (28%)
3. Did you receive an explanation about deafness gene and hereditary hearing loss from a physician?	
Yes	103/134 (77%)
No	31/134 (23%)
	($p < 0.001$)
4. Those who answered "Yes" to the above question: Did you understand the contents of the explanation?	
Well understood	26/103 (25%)
Tolerably understood	62/103 (60%)
Not understood	14/103 (14%)
No response	1/103 (1%)
5. Those who answered "Well understood" or "Tolerably understood" to the above question: Did you change your attitude towards your own hearing loss after receiving the explanation?	
Yes	21/88 (24%)
No	48/88 (54%)
Not sure	19/88 (22%)
6. When you heard that your hearing loss has a possibility to be caused by a genetic mutation, did you want to receive the genetic testing?	
Yes	83/136 (61%)
No	2/136 (1.5%)
Not sure	51/136 (37.5%)
7. About the genetic testing that you received	
I was willing to receive it.	53/135 (39%)
I received it on a physician's advice.	70/135 (52%)
I received it on a family member's advice.	2/135 (1.5%)
I did not want to receive it.	10/135 (7.5%)
8. If the opportunity arose, do you want to participate in other genetic studies in the future?	
I will positively participate in them.	44/137 (32%)
I will participate in them by condition.	81/137 (59%)
I will not participate in them.	12/137 (9%)
9. This hospital has an outpatient department of medical genetics that can offer genetic counseling. Did you know the existence of this outpatient department or of genetic counseling?	
Medical genetics department	
Yes	10/138 (7%)
No	128/138 (93%)
	($p < 0.001$)
Genetic counseling	
Yes	7/134 (5%)
No	127/134 (95%)
	($p < 0.001$)

Table 1 (*Continued*)

Questions and arranged answers	Proportion (%) (p value)
10. Those who answered "yes" to the above question: How did you learn about it?	
By a physician's explanation	12/12 (100%)
Found out by myself	0/12 (0%)
11. Do you want to visit the department and receive counseling?	
Yes	41/132 (31%)
No	11/132 (8%)
Not sure	75/132 (57%)
Already visited and received counseling	5/132 (4%)
12. Those who answered "yes" to the above question: What do you most want from the department? Check all that apply.	
Cause of hearing loss	25 (61%)
Pattern of inheritance	25 (61%)
Own future	22 (54%)
Children's future	23 (56%)
Marriage	6 (15%)
Informing your family	3 (7%)
Other	4 (10%)
13. Those who were told about the results of having any deafness gene mutation: Were you worried about having the mutation?	
Yes, very much	3/8 (37.5%)
Yes, a little	2/8 (25%)
No	3/8 (37.5%)
14. Those who were told about the results of having any deafness gene mutation: Were you involved with social and/or family problems after being proved to have such a mutation?	
Yes	0/8 (0%)
No	7/8 (87.5%)
Might be involved in the future	1/8 (12.5%)

answers varied from 132 to 138, because some respondents left some blanks in response to some of the questions. Question numbers 1–5 were based on a "deafness gene and hereditary HL". Before visiting our department and receiving the genetic testing, 88 (64%) of 138 respondents were unaware that a genetic factor can cause HL. Meanwhile, 50 (36%) of 138 respondents who were aware of the causative effect of a genetic factor had obtained the information from various sources including another hospital, a friend, a magazine, and the Internet. One hundred and three (77%) of the 134 respondents answered that they had received an explanation about a deafness gene and hereditary HL from a physician who belonged to our department. Out of the 103 respondents, 88 respondents (85%) answered that they understood the contents well or tolerably, whereas 14 (14%) respondents answered that they did not understand. Twenty-one (24%) of the 88 respondents who answered "well or tolerably understood" changed their attitude towards HL.

3.2. Genetic testing

The question numbers 6–8 were related to "genetic testing". When patients were informed about the possibility of having a genetic mutation, 83 (61%) of 136 respondents indicated that they would like to receive the genetic testing, 51 (37.5%) answered "not sure", and 2 (1.5%) respondents did not wish to receive the tests. At the time of the response to the questionnaire, the frequency of the number of respondents who did not wish to receive the genetic testing increased to 7.5%. Meanwhile, 39% of the respondents received the tests positively, and the remaining 53.5% received it upon the advice of a physician or family member. With respect to future participation in other genetic tests, 91% of the respondents answered in the affirmative whereas 9% answered in the negative.

3.3. Outpatient department of medical genetics/genetic counseling

In question numbers 9–12, we asked about "the outpatient department of medical genetics/genetic counseling". Although our acceptance form provided the information on the outpatient department and the genetic counseling, more than 90% of the respondents remained unaware of their existence. All respondents who were aware had obtained the information from a physician of our department. When the respondents learnt of this information, nearly one third (31%) of the respondents wanted to visit the outpatient department and receive the genetic counseling, but 8% of the respondents did not wish to do so. Five respondents had already visited the department of medical genetics and had received the genetic counseling; one respondent received the genetic counseling at our facility and the remaining four respondents did so at the other facilities. The respondents wished to learn about the cause of HL, the patterns of inheritance, their own and their children's future.

3.4. Results of genetic testing

The question numbers 13 and 14 were related to the results of genetic testing. Eight patients who had mutations in a deafness gene answered these questions. Five respondents answered that they worried about having a mutation, while the remaining 3 were not worried about it. No respondent answered that they had social or family problems.

3.5. Statistical analysis

A significant difference was observed (question number 1: $p < 0.05$, question numbers 3 and 9: $p < 0.01$) in regard to the yes-or-no answers (Table 1).

4. Discussion

According to the recent result of the newborn hearing screening conducted in Colorado State, one in 650 infants had HL [2]. About half of the cases of congenital deafness or profound HL in early childhood are caused by a single gene mechanism [1]. The provision of genetic testing to patients

with congenital deafness and/or familial HL increases the chances of finding the deafness genes. Therefore, we need to consider the ethical issues of genetic testing and to understand the attitude of patients towards genetic testing and HL. There have been several reports related to this matter [3–7]. However, it remains possible that the opinions and attitudes towards genetic testing and genetics are different among various ethnic groups. Thus, we conducted the present attitude survey.

In the present study, more than half (64%) of the respondents who received the genetic testing for HL were not aware that HL can be caused by a genetic factor. It is probably because we included patients with idiopathic bilateral sensorineural HL in the study. The reason why we performed the genetic study for idiopathic bilateral progressive sensorineural HL is that mitochondrial DNA 1555A>G is a frequent mutation among the Japanese population and is seen even in HL patients whose family members can hear [8]. According to a survey of normal hearing parents who had one or more deaf children, the majority of responders had a poor understanding of their risk of having another deaf child and their deaf child's chance of having future deaf children [4]. In the present study we intended to provide full information about the deafness gene, the concept of inheritance, and the risks, benefits, and limitations of genetic testing. However, about one fourth (23%) of the respondents answered that they had not received an explanation of a deafness gene. Moreover, 14% of the respondents who answered that they had received the explanation did not understand the explanation. Although it may be difficult for patients to understand genetics correctly, we should try to provide a clearer understanding to them. In addition, about one forth (24%) of the respondents who answered that they could well or tolerably understand a deafness gene and hereditary HL changed the attitude towards their own HL.

When patients learned of the possibility of having a genetic mutation in the outpatient clinic, 37.5% of respondents were not certain that they would like to receive the genetic testing. At the time of the response to the questionnaire, 53.5% of responders received genetic testing upon the advice of a physician or family member. These results suggested that about half of patients may not make their own decision regarding genetic testing. At the time of the response to the questionnaire, the frequency of the number of respondents who did not wish to receive the genetic testing increased to 7.5%. This is partly because most of the patients who wished to know the etiology of their own HL and/or to confirm the absence of deafness gene mutations could not obtain any results.

There is a substantial difference in attitude towards genetic testing for HL between deaf adults and hearing adults with deaf children. Middleton et al. reported that deaf adults had a predominantly negative attitude towards genetic testing and 55% of the individuals thought genetic testing to be more harmful than beneficial [3]. On the other hand, the vast majority of the hearing parents with deaf children had an overall positive attitude towards genetic testing [4,6]. In the present study, if an opportunity presented itself, 125 of 137 respondents wished to participate in other genetic studies in the future. This suggested that the majority (91%) of responders had a positive attitude towards genetic testing. With respect to the degree of satisfaction for genetic testing in other disorders, 81% of the individuals who had one or more first-degree relatives affected with breast cancer planned to seek genetic testing [9], and 95.1% of parents of children with cystic fibrosis (CF) or patients with CF favored a prenatal diagnosis [10].

Genetic counseling provides appropriate information regarding genetics to patients and/or their family members (clients) to allow the clients to have their own preferred life plans. Furthermore, a genetic counselor needs to give moral support to the clients while maintaining a favorable and confidential relationship with them. Paker et al. reported that 34.6% of parents with hearing impaired children received genetic counseling from a recognized clinical genetic service and approximately half of the parents who were not offered any genetic counseling answered they would like to have received this option [5]. However, the respondents who received it made comments that were equally positive and negative towards the counseling. In this study, only less than 10% knew the availability of the genetic outpatient department and counseling and only five respondents received genetic counseling from a recognized genetic outpatient department, despite the fact that the information was written in the consent form. Meanwhile, 31% of the respondents would have liked to visit the department and to receive genetic counseling, when they learned of the availability of these resources. Although we should pay attention to the patients who have a negative attitude to genetic counseling, we need to provide more information about the genetic outpatient department and counseling. The most important reasons for receiving genetic testing were reported as determining the etiology of HL, and its risk of recurrence [4–6]. Our findings that the most common requests for genetic counseling were the cause of HL, patterns of inheritance, the individuals' own and their children's future, were consistent with those of previous papers.

From our point of view, detecting a causative mutation is useful for determining the etiology and prognosis of a disease, its repetition rate, preventive and therapeutic measures, and genetic counseling. The collection rate was 70% in the present attitude survey, and thus there may have been some bias in the results. However, the satisfaction level with genetic testing for HL is high in many cases. In the present study, no respondents who were told about having any deafness gene mutation developed social and/or family problems. However, some respondents were worried about the results. Therefore, we should fully recognize that relaying genetic information to a family appears to be justifiable for physicians, but may be burdensome to the patients [11].

5. Conclusion

We conducted a questionnaire survey to investigate the patients' understanding and satisfaction regarding genetic testing for HL. The results showed a high satisfaction level for the genetic testing. The common requests for the genetic counseling were the etiology of HL, patterns of inheritance, the individuals' own and their children's future, which were consistent with previous reports. However, 7.5% of the respondents regretted receiving the genetic testing and some respondents were worried about the results of the genetic testing. Therefore, we should bear in mind that there are patients who have a negative attitude towards genetic testing and may become burdened by the results.

Acknowledgements

We thank all of the family members who participated in this study. This study was supported in part by Grants-in-Aid for Scientific Research (Nos. 19591960, 15790924, 14370539, 16659462, 16012215) from the Ministry of Science, Education, Sports, and Culture of Japan, and by Health and Labour Sciences Research Grants (H13-006; Researches on Sensory and Communicative Disorders, and H14-21 and No. 17242101; Research on Measures for Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan.

References

[1] Morton NE. Genetic epidemiology of hearing impairment. Ann N Y Acad Sci 1991;630:16–31.
[2] Mehl AL, Thomson V. The Colorado newborn hearing screening project, 1992–1999: on the threshold of effective population-based universal newborn hearing screening. Pediatrics 2002;109:E7.
[3] Middleton A, Hewison J, Mueller RF. Attitudes of deaf adults toward genetic testing for hereditary deafness. Am J Hum Genet 1998;63:1175–80.
[4] Brunger JW, Murray GS, O'Riordan M, Matthews AL, Smith RJ, Robin NH. Parental attitudes toward genetic testing for pediatric deafness. Am J Hum Genet 2000;67:1621–5.
[5] Paker MJ, Fortnum HM, Young ID, Davis AC. Genetics and deafness: what do families want? J Med Genet 2000;37:E26.
[6] Dagan O, Hochner H, Levi H, Raas-Rothschild, Sagi M. Genetic testing for hearing loss: different motivations for the same outcome. Am J Med Genet 2002;113:137–43.
[7] Guillemin M, Gillam L. Attitudes to genetic testing for deafness: the importance of informed choice. J Genet Couns 2006;15:51–9.
[8] Noguchi Y, Yashima T, Ito T, Sumi T, Tsuzuku T, Kitamura K. Audiovestibular findings in patients with mitochondrial A1555G mutation. Laryngoscope 2004;114:344–8.
[9] Jacobsen PB, Valdimarsdottier HB, Brown KL, Offit K. Decision-making about genetic testing among women at familial risk for breast cancer. Psychosom Med 1997;59:459–66.
[10] De Braekeleer M, Rault G, Bellis G. Reproductive attitudes of couples having a child with cystic fibrosis in Brittany (France). J Hum Genet 2004;49:285–9.
[11] Forrest K, Simpson SA, Wilson BJ, Teijlingen ER, McKee L, Haites N, et al. To tell or not to tell: barriers and facilitators in family communication about genetic risk. Clin Genet 2003;64: 317–26.

Acta Oto-Laryngologica, 2010; 130: 1002–1008

informa
healthcare

ORIGINAL ARTICLE

Novel *ATP6V1B1* mutations in distal renal tubular acidosis and hearing loss

TAKATOSHI YASHIMA[1], YOSHIHIRO NOGUCHI[1], YOSHIYUKI KAWASHIMA[1], TATEMITSU RAI[2], TAKU ITO[1] & KEN KITAMURA[1]

[1]*Department of Otolaryngology and* [2]*Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan*

Abstract

Conclusion: Novel *ATP6V1B1* mutations were found in a patient with distal renal tubular acidosis (dRTA), hearing loss (HL), and enlargement of the vestibular aqueduct (EVA). The deterioration of HL and vertiginous attacks may be associated with the disruption of the endolymph pH homeostasis. *Objectives:* To study the audiovestibular functions and to identify the causative gene. *Methods:* This study enrolled a Japanese family, where the proband showed type 1 dRTA, early onset HL, and bilateral EVA. A deterioration of HL occurred several times in both ears. Vertiginous attacks were always associated with a deterioration of HL. Audiovestibular examinations included distortion product otoacoustic emissions (DPOAEs), auditory brainstem responses (ABRs), caloric testing, and vestibular evoked myogenic potentials (VEMPs). Direct sequencing was utilized to screen for *ATP6V1B1*, *SLC26A4*, and *GJB2* mutations. *Results:* The findings of DPOAEs and ABRs indicated cochlear HL. The vestibular function was thought to be mildly impaired according to the caloric responses and VEMP findings. Two novel *ATP6V1B1* mutations of a heterozygous 15 base-pair deletion (c.756_770del) in exon 7 and a heterozygous 1 base-pair insertion (c.1242_1243insC) in exon 12 were detected in a compound heterozygous state. No mutation was identified in either *SLC26A4* or *GJB2*.

Keywords: *Deafness, vertigo, vestibular evoked myogenic potentials, enlargement of vestibular aqueduct, large vestibular aqueduct*

Introduction

Renal tubular acidosis (RTA) is a heterogeneous disorder characterized by normal anion gap metabolic acidosis in spite of either a normal or near-normal glomerular filtration rate [1]. This disorder is caused by tubular defects in urinary acidification involving either the distal nephron (type 1 distal RTA) or the proximal nephron (type 2 proximal RTA). Proximal RTA results from a failure of the reabsorption of bicarbonate (HCO_3^-) in the proximal nephron, whereas distal RTA results from a failure of the secretion of hydrogen ions (H^+) in the distal nephron, either of which may be inherited or acquired. Moreover, there is combined proximal and distal RTA (type 3 RTA), and hyperkalemic distal RTA (type 4 RTA) [1].

Type 1 distal RTA (dRTA) is the classical form of RTA and it can cause hyperchloremic metabolic acidosis, hypokalemia, nephrocalcinosis, and renal calculi. The association of dRTA and hearing loss (HL) was first noted in 1967 [2], and since that time several patients with this clinical condition have been reported [3,4]. Recently, the identification of the causative genes for dRTA has provided considerable progress in understanding the pathophysiology of this group of disorders [1]. Mutations in *SLC4A1* that encodes AE1, the Cl^-/HCO_3^- exchanger on the basolateral membrane of the α-intercalated cell of the distal nephron, can cause either autosomal dominant

Correspondence: Yoshihiro Noguchi MD PhD, Department of Otolaryngology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. Fax: +81 3 3813 2134. E-mail: noguchi.oto@tmd.ac.jp

(Received 28 November 2009; accepted 14 January 2010)

ISSN 0001-6489 print/ISSN 1651-2251 online
DOI: 10.3109/00016481003631529

or recessive dRTA without HL. Meanwhile, an H^+-adenosine triphosphatase (ATPase) in the apical border of the α-intercalated cell of the distal nephron is a multi-subunit structure that plays a role in pumping H^+ into the tubular lumen. To date, two genes (*ATP6V1B1* and *ATP6V0A4*) that encode the H^+-ATPase have been identified to be responsible for dRTA. Mutations in *ATP6V1B1*, which encodes the B1-subunit of H^+-ATPase, can cause dRTA and early onset HL, whereas mutations in *ATP6V0A4*, which encodes the a4-subunit of H^+-ATPase, can cause dRTA with either normal hearing or late onset hearing loss (HL) [5,6].

The enlargement of the vestibular aqueduct (EVA) is the most common malformation of the inner ear, and mutations of several deafness genes have been reported to be responsible for EVA. *SLC26A4* mutations are a common cause of EVA in Western populations as well as those in East and South Asian populations [7]. *EYA1* and *SIX1* mutations are responsible for branchio-oto-renal/branchio-oto syndrome (BOS) that can be associated with EVA [8,9]. Furthermore, a recent study demonstrated that *ATP6V1B1* mutations could be responsible for bilateral EVA [10]. The present study reports additional mutations of *ATP6V1B1* in a patient with dRTA, early onset HL, vertiginous attack, and bilateral EVA. Audiovestibular testing, including vestibular evoked myogenic potentials (VEMPs) was performed to investigate the pathophysiology of this disorder.

Material and methods

Patients

This study enrolled a non-consanguineous Japanese family, in which the proband suffered from dRTA and bilateral mixed HL (Figure 1) with normal tympanic membranes. All family members with the exception of the proband had no dRTA, while the parents showed bilateral sensorineural HL (Figure 1). The HL of the father was thought to be noise-induced HL, because he showed bilateral C^5-dip, and is employed in the construction business where he experiences continuous noise exposure. An audiogram of the mother showed bilateral mid-frequency sensorineural HL. She presented with non-progressive HL from childhood. Two younger brothers of the proband had no HL according to an interview with the mother.

Case report

The proband was a 12-year-old male when he first visited the Department of Otolaryngology, Tokyo Medical and Dental University Hospital in March 2001. There were no abnormal events throughout gestation and at birth, while his body weight did not increase well due to his poor suckling. Renal calcification was found at his 1 month medical screening, and he was diagnosed with dRTA. Alkali therapy was initiated to treat the acidosis. By the age of 3 years, it was obvious that the patient had developed HL, and thus he began to use bilateral hearing aids. There had been several attacks of rotatory vertigo since the age of 4, but the HL had not shown any apparent exacerbation.

Figure 2 shows the clinical course of pure-tone averages (PTAs) calculated from the audiometric thresholds at the frequencies of 500, 1000, and 2000 Hz, the attacks of vertigo, and the value of potassium after the first visit to the Department of Otolaryngology. He needed to be hospitalized three times between the ages of 12 and 14 for hypokalemia attacks that were difficult to control. Blood examinations showed metabolic acidosis with a normal anion gap, and the urinary pH showed a defect in urinary

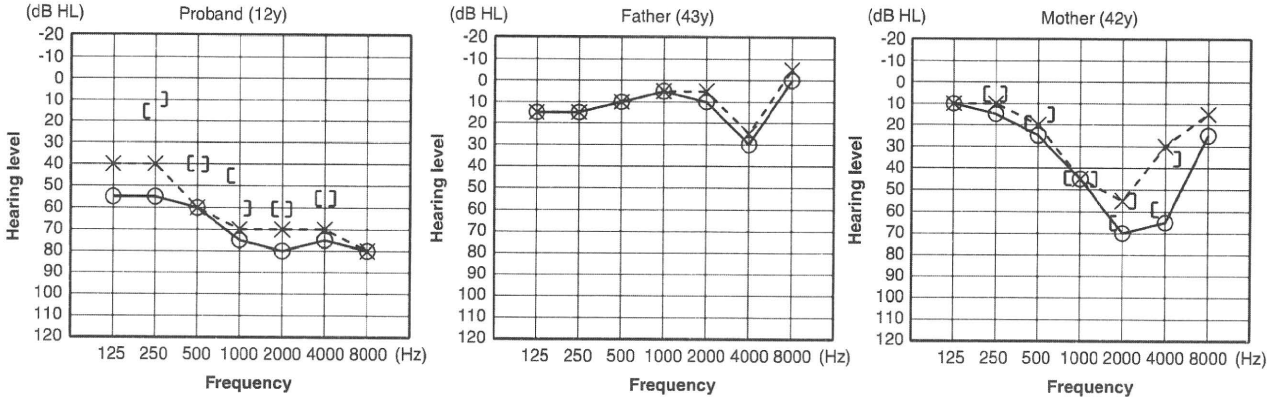

Figure 1. Audiograms of three family members. Bilateral air–bone gaps are recognized, especially at lower frequencies in the proband in July 2001.

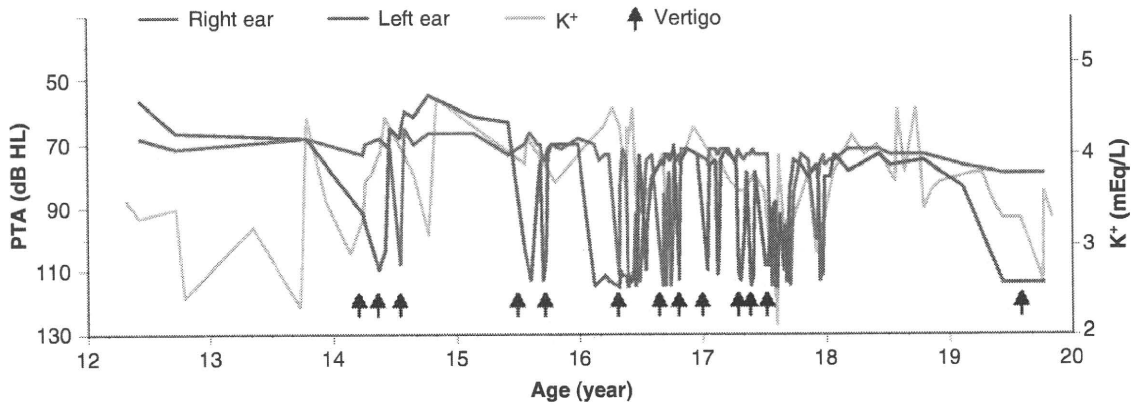

Figure 2. Clinical course of pure-tone averages (PTAs), attacks of vertigo, and the value of potassium (K^+). PTA is calculated from the audiometric thresholds at the frequencies of 500, 1000, and 2000 Hz.

acidification (Table I). He experienced hypokalemia that was difficult to control between the ages of 13 and 14. Thereafter, fluctuating HL and vertiginous attacks started. An exacerbation of HL occurred 7 times in the right ear and 14 times in the left ear during the follow-up period. One of these exacerbations occurred after mild head trauma, and another exacerbation happened after a common cold infection. He received intravenous corticosteroids at each exacerbation, with careful attention to his electrolyte disorder. Hyperbaric oxygen was administered as an additional therapy when the corticosteroid therapies were not effective. There were 12 attacks of vertigo that were always associated with the exacerbation of HL and nausea, and lasted for several hours. However, the exacerbation of HL was not necessarily associated with hypokalemia and the alkali therapy was not effective for the exacerbation of HL. From the age of 12 to the age of 19, his right PTA was maintained from 71.7 dB to 68.3 dB in remission, but his left PTA deteriorated from 56.7 dB to complete deafness at the age of 19, and showed no subsequent recovery.

High-resolution computed tomography (CT) and magnetic resonance imaging (MRI) of the temporal bones showed bilateral EVA with no other inner and middle ear malformations (Figure 3).

Table I. Laboratory data of the proband at the first hospitalization.

Parameter		Value	Normal range
Blood	pH	7.322	7.35–7.45
	BE	–4.0	–2.0 to 2.0
	HCO_3^- (mEq/l)	21.5	22.0–26.0
	Na^+ (mEq/l)	144	138–146
	K^+ (mEq/l)	3.3	3.7–5.0
	Cl^- (mEq/l)	111	99–107
	Anion gap (mEq/l)	11.5	10.0–14.0
Urine	pH	7.5	5.0–7.5

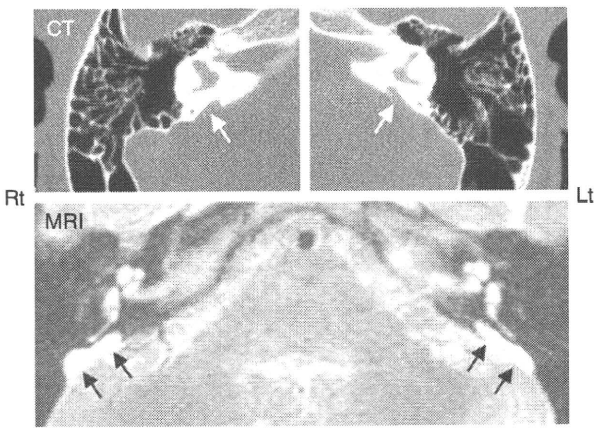

Figure 3. High-resolution computed tomography (CT) and magnetic resonance imaging (MRI) of the temporal bones in the proband. Bilateral enlargement of the vestibular aqueduct and endolymphatic sac (arrows) is evident.

Audiovestibular examinations

The proband underwent audiological examinations that included distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs). DPOAEs were recorded and analyzed using an ILO292 analyzer (Otodynamics Ltd, Hatfield, Herts, UK). Two primary tones with a frequency ratio (f2/f1) kept at 1.22 were presented at 70 dB sound pressure level. Alternating polarity clicks at the intensity of 90 dB normal hearing level (nHL) were presented as the acoustic stimuli at a frequency of 9.5 Hz for the measurement of ABRs. The inter-peak latency difference between waves I and V (IPL I–V)

was used as a criterion and an IPL I–V of longer than 4.4 ms was considered to be abnormal.

The vestibular examinations, including gaze, positional, positioning and spontaneous nystagmus testing, and air caloric testing were performed using electronystagmography (ENG). Caloric hypoplexia was defined as a maximal slow phase velocity (MSV) <20°/s. VEMPs were measured using a previously described procedure [11]. In brief, short tone-bursts with a frequency of 500 Hz were delivered from headphones at a stimulus repetition rate of 5 Hz. The stimulus intensities were configured as 95 and 105 dB nHL.

Mutation analysis

All protocols were approved by the Ethics Reviewing Committee of Tokyo Medical and Dental University and were carried out only after obtaining written informed consent. Genomic DNA was extracted from the peripheral blood lymphocytes of the patient and his parents. Unfortunately, genomic DNA could not be obtained from the two younger brothers of the proband. The coding regions and flanking intronic sequences of *ATP6V1B1* were amplified using a combination of previously described primer pairs [5]. However, newly designed pairs (forward: 5′-CT GGTCAGTCCACAGGCTTT-3′, reverse 5′-TGG CTGTAGTTCTGGGATGC-3′) were used for exon 8. Amplification was also conducted for the coding regions of *SLC26A4* that is the most prevalent causative gene of EVA, and *GJB2*, the most frequent causative gene of non-syndromic hereditary HL. PCR was carried out in a 50 µl volume containing 50 ng of DNA, 0.6 µmol/L each primer, 200 µmol/L each dNTP, 5 µl of 10× QIAGEN PCR buffer, and 1.25 U of *Taq* DNA polymerase (*Taq* PCR Core Kit, QIAGEN) in a GeneAmp PCR System 9700 (PE Applied Biosystems). The amplified products were purified using a QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer's instructions and then were directly sequenced using an Applied Biosystems Prism BigDye Terminator Cycle Sequencing Ready Kit and an ABI Prism model 310 Genetic Analyzer.

Results

Audiovestibular findings

DPOAEs, ABRs, and ENG were performed in December 2003, when the PTA was 66.7 dB in the right ear and 61.7 dB in the left ear. The DPOAE amplitudes decreased to the noise levels bilaterally. In the ABR measurements, no waveform was seen on the right side, while the IPL I–V was not prolonged on the left side. These findings suggested the presence of a cochlear dysfunction without any retrocochlear dysfunction. No gaze, positional, positioning, and spontaneous nystagmus were detected. The MSV of caloric testing was 14°/s in the right ear and 17°/s in the left ear, thus suggesting bilateral slight caloric hypoplexia.

VEMPs were carried out in February 2006, when the PTA of the right ear was 73.3 dB and the left ear showed complete deafness. In the right ear, clear waveforms of VEMPs were recognized at both stimulus intensities of 95 and 105 dB nHL, while in the left ear, VEMPs were absent at 95 dB nHL, but they were detected at 105 dB nHL (Figure 4).

Genetic findings

Two novel *ATP6V1B1* mutations of a heterozygous 15 bp deletion (c.756_770del) in exon 7 and a heterozygous 1 bp insertion (c.1242_1243insC) in exon 12 were detected in the proband (Figure 5). The father and mother of the proband had a heterozygous c.1242_1243insC and a heterozygous c.756_770del, respectively. These mutations were not found in 80 normal controls. Therefore, it is possible that the compound heterozygous mutations caused dRTA and HL in the proband. Four previously reported polymorphic changes were found, including c.89T>C (M1T), c.114T>C (P9P), c.176C>T (T30I), and c.1089C>T (R334R).

No mutation was detected in either *SLC26A4* or *GJB2* in the proband. However, a previously reported

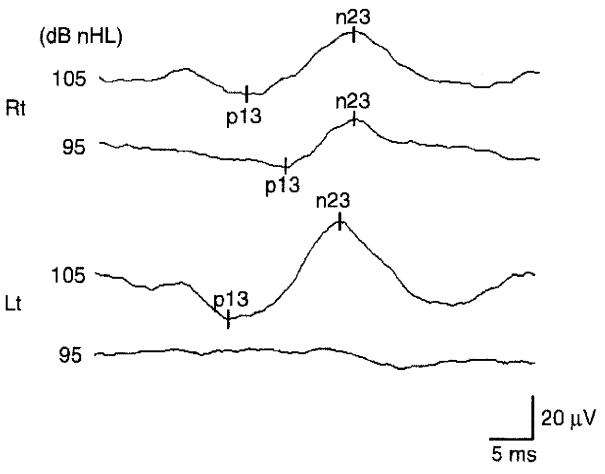

Figure 4. Vestibular evoked myogenic potentials (VEMPs) in the proband. The first positive-negative peaks (p13-n23) are shown in the waveforms. Clear waveforms are seen at both stimulus intensities of 95 and 105 dB nHL in the right ear, while a positive response is obtained only at 105 dB nHL in the left ear.

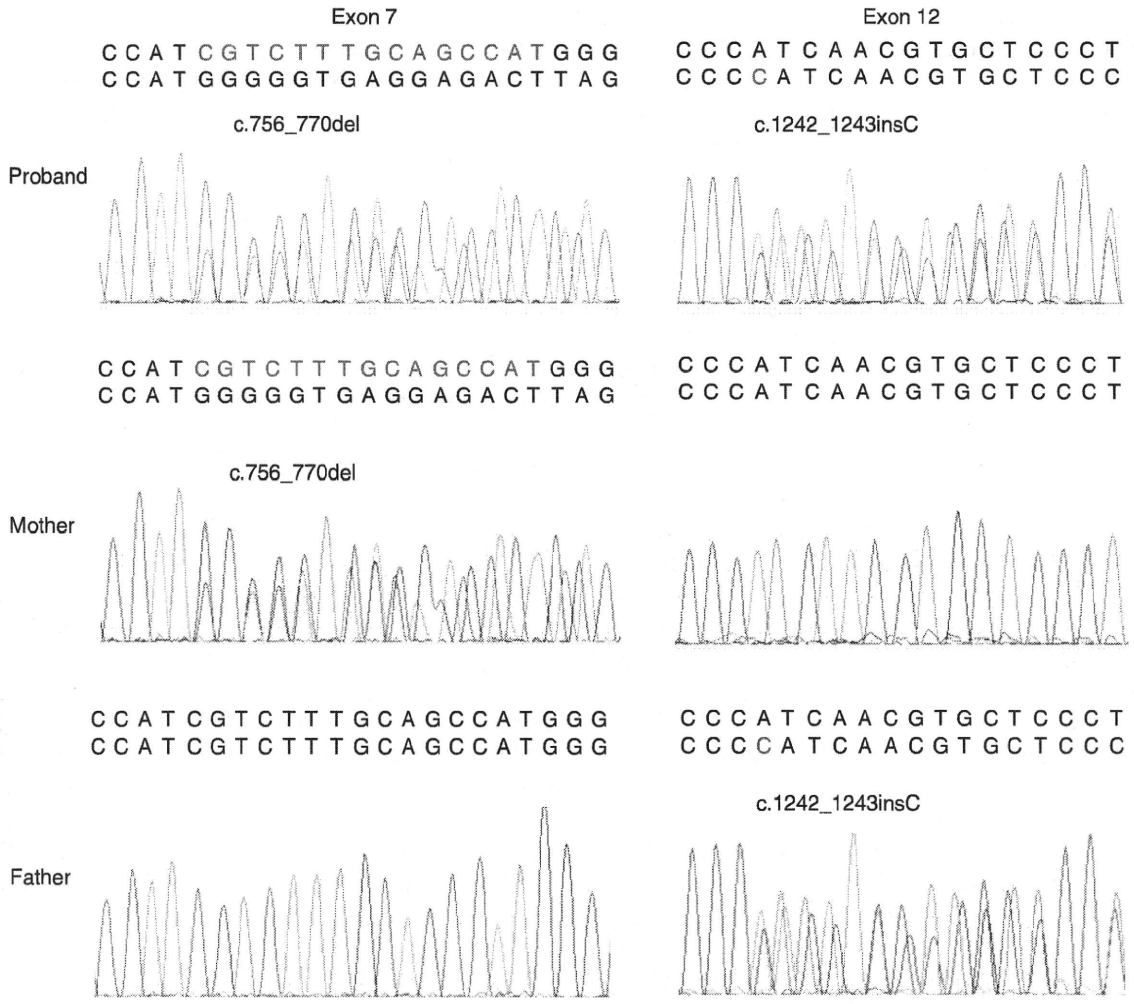

Figure 5. Electropherograms in the family members. Compound heterozygous *ATP6V1B1* mutations of a heterozygous 15 bp deletion (c.756_770del) in exon 7 and a heterozygous 1 bp insertion (c.1242_1243insC) in exon 12 were detected in the proband.

polymorphic change of c.79A>G (V27I) in *GJB2* was found.

Discussion

Nearly 30 mutations in *ATP6V1B1* have so far been detected from subjects with various ethnic backgrounds and dispersed throughout the coding regions and splice sites of the gene [5,6,10,12–15]. More than half of the mutations are missense mutations followed by 1 bp deletions or insertions, splice-site mutations, and nonsense mutations. The present study detected two novel mutations of a 15 bp deletion (c.756_770del) and 1 bp insertion (c.1242_1243insC) in the proband with typical phenotypes caused by *ATP6V1B1* mutations. Neither of the mutations was detected in 80 normal controls. Moreover, no mutation was found in *SLC26A4*. Therefore, c.756_770del and c.1242_1243insC were disease-causing mutations. The combination of c.756_770del and c.1242_1243insC were predicted to lead to defects in the protein structure and/or function, because neither of these mutations led to frameshift and a premature stop codon.

The parents of the current proband also had HL. The HL in the father with a heterozygous c.1242_1243insC was thought to be noise-induced HL. Meanwhile, the mother with a heterozygous c.756_770del showed bilateral mid-frequency sensorineural HL. The dominant-negative effect by the heterozygous mutation may cause HL but not dRTA. However, the true etiology of the HL is unknown, because the parents of the mother and two younger brothers of the proband, who might have a deletion mutation, had normal hearing according to the interview with the mother.

The pH of the endolymph varies in the different regions in the inner ear, and endolymph pH

homeostasis is necessary for hearing and the prevention of HL [16]. *ATP6V1B1* mRNA is expressed in both the fetal and adult cochlea [5]. Furthermore, the product of the gene, B1-subunit of H^+-ATPase, is strongly observed in the interdental cell layer of the spiral limbus and the epithelial cells of the endolymphatic sac of mice [4]. Both the interdental cells and endolymphatic cells come in direct contact with the endolymph. Therefore, the loss of the B1-subunit of H^+-ATPase function may cause a disruption of the endolymph pH homeostasis, thus resulting in HL.

So far there have been three reports that noted an association between dRTA and EVA [10,17,18]. Berrettini et al. [17] reported three patients with dRTA and HL who showed either unilateral or bilateral EVA. Shinjo et al. [18] also reported a patient who had dRTA, HL, and bilateral EVA. However, the previous four patients did not undergo any genetic studies. Recently, Joshua et al. [10] showed that four patients from three families with homozygous *ATP6V1B1* mutations had dRTA and HL as well as bilateral EVA. The HL started in early childhood, and was asymmetrical, moderate to severe, and progressive. The present study confirmed that *ATP6V1B1* could therefore be responsible for EVA.

Although the proband demonstrated no middle ear abnormalities according to the otoscopic examination and CT findings, pure-tone audiometry showed bilateral air–bone gaps, especially at the lower frequencies. The air–bone gap is often recognized in patients with EVA. There have been several explanations for the mechanism, and a recent report speculated that EVA acting as a third mobile window in the inner ear resulted in the air–bone gap [19].

No previous report has disclosed that patients with *ATP6V1B1* mutations show fluctuating HL and vertiginous attacks. The deterioration of HL in the current proband occurred several times in each ear and recovered within a few months every time with the exception of the last exacerbation in the left ear. Repetitive vertiginous attacks occurred with the combination of the deterioration of HL, whereas vestibular function was not severely impaired according to the vestibular examinations. Taken together, the deterioration of audiovestibular function in this disorder is thought to be reversible to a certain extent. The same clinical course in audiovestibular impairment was seen in a patient with Pendred syndrome that was caused by an *SLC26A4* mutation and showed EVA [20]. However, it is non-obligatory in patients with BOS that can also show EVA [8,9]. Therefore, EVA may not be the major cause of the deterioration of audiovestibular function. Meanwhile, the endolymph pH homeostasis depends on the secretion of H^+ as well as HCO_3^-, and HCO_3^- may be secreted into the endolymph via the HCO_3^- permeable anion exchanger pendrin, the product of *SLC26A4* [16]. Therefore, the disruption of the endolymph pH homeostasis may cause an exacerbation of the audiovestibular function in patients with *ATP6V1B1* or *SLC26A4* mutations. However, the exacerbations of the audiovestibular function in this case occurred even when the value of potassium was within the normal range. Therefore, general medical conditions may not necessarily affect the state of endolymph pH homeostasis.

Alkali therapy was not effective for the treatment of HL in the proband, which was also consistent with a previous report [4]. Intravenous corticosteroid therapy with and without hyperbaric oxygen was performed every time the HL of the proband deteriorated. It is not known whether these treatments are truly effective for the deterioration of HL, because a natural recovery of hearing without any special treatment can occur in patients with EVA. However, a few reports have recommended either corticosteroid therapy [21] or hyperbaric oxygen [22] for the treatment of hearing deterioration associated with EVA. Therefore, the early diagnosis, close monitoring of hearing, avoidance of head trauma, and appropriate treatments are all essential for patients with dRTA, HL, and EVA to maintain their hearing ability.

Conclusions

Two novel *ATP6V1B1* mutations of a heterozygous 15 bp deletion and a heterozygous 1 bp insertion were detected in a patient with type 1 dRTA, HL, and EVA. Mutations in *ATP6V1B1* may thus cause a disruption of the endolymph pH homeostasis, resulting in both a worsening of HL and the onset of vertiginous attacks.

Acknowledgments

We thank all of the family members who participated in this study. The study was supported in part by Grants-in-Aid for Scientific Research (nos 19591960, 15790924, 14370539, 16659462, 16012215) from the Ministry of Health, Labour and Welfare of Japan, and by Health and Labour Sciences Research Grants (H13-006; Researches on Sensory and Communicative Disorders, and H14-21 and no. 17242101; Research on Measures for Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Fry AC, Karet FE. Inherited renal acidosis. Physiology 2007;22:202–11.

[2] Royer P, Broyer M. 1967. L'acidose rénale au cours des tubulopathies congénitales. In: Proceedings of Actualités Néphrologiques de l'Hôpital Necker. Paris: Flammarion. p 73–92.

[3] Brown MT, Cunningham MJ, Ingelfinger JR, Becker AN. Progressive sensorineural hearing loss in association with distal renal tubular acidosis. Arch Otolaryngol Head Neck Surg 1993;119:458–60.

[4] Zakzouk SM, Sobki SH, Mansour F, al Anazy FH. Hearing impairment in association with distal renal tubular acidosis among Saudi children. J Laryngol Otol 1995;109:930–4.

[5] Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, et al. Mutations in the gene encoding B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. Nat Genet 1999;21:84–90.

[6] Stover EH, Borthwick KJ, Bavalia C, Eady N, Fritz DM, Rungroj N, et al. Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. J Med Genet 2002;39: 796–803.

[7] Park HJ, Shaukat S, Liu XZ, Hahn SH, Naz S, Ghosh M, et al. Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness. J Med Genet 2003;40:242–8.

[8] Kemperman MH, Koch SM, Joosten FB, Kumar S, Huygen PL, Cremers CW. Inner ear anomalies are frequent but nonobligatory features of the branchio-oto-renal syndrome. Arch Otolaryngol Head Neck Surg 2002;128: 1033–8.

[9] Ito T, Noguchi Y, Yashima T, Kitamura K. SIX1 mutation associated with enlargement of the vestibular aqueduct in a patient with branchio-oto syndrome. Laryngoscope 2006;116: 796–9.

[10] Joshua B, Kaplan DM, Raveh E, Lotan D, Anikster Y. Audiometric and imaging characteristics of distal renal tubular acidosis and deafness. J Laryngol Otol 2008;22:193–8.

[11] Kawashima Y, Noguchi Y, Ito T, Kitamura K. Vestibular evoked myogenic potentials in patients with mitochondrial A1555G mutation. Laryngoscope 2009;119:1874–9.

[12] Vargas-Poussou R, Houillier P, Le Pottier N, Strompf L, Loirat C, Baudouin V, et al. Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. J Am Soc Nephrol 2006;17:1437–43.

[13] Ruf R, Rensing C, Topaloglu R, Guay-Woodford L, Klein C, Vollmer M, et al. Confirmation of the ATP6B1 gene as responsible for distal renal tubular acidosis. Pediatr Nephrol 2003;18:105–9.

[14] Hahn H, Kang HG, Ha IS, Cheong HI, Choi Y. ATP6B1 gene mutations associated with distal renal tubular acidosis and deafness in a child. Am J Kidney Dis 2003;41:238–43.

[15] Gil H, Santos F, García E, Alvarez MV, Ordóñez FA, Málaga S, et al. Distal RTA with nerve deafness: clinical spectrum and mutational analysis in five children. Pediatr Nephrol 2007;22:825–8.

[16] Lang F, Vallon V, Knipper M, Wangemann P. Functional significance of channels and transporters expressed in the inner ear and kidney. Am J Physiol Cell Physiol 2007;293: C1187–1208.

[17] Berrettini S, Forli F, Franceschini SS, Ravecca F, Massimetti M, Neri E. Distal renal tubular acidosis associated with isolated large vestibular aqueduct and sensorineural hearing loss. Ann Otol Rhinol Laryngol 2002;111:385–91.

[18] Shinjo Y, Kaga K, Igarashi T. Distal renal tubular acidosis associated with large vestibular aqueduct and sensorineural hearing loss. Acta Otolaryngol 2005;125:667–70.

[19] Merchant SN, Nakajima H, Halpin C, Nadol JB Jr, Lee DJ, Innis WP, et al. Clinical investigation and mechanism of airbone gaps in large vestibular aqueduct syndrome. Ann Otol Rhinol Laryngol 2007;116:532–51.

[20] Iwasaki S, Usami S, Abe S, Isoda H, Watanabe T, Hoshino T. Long-term audiological feature in Pendred syndrome caused by PDS mutation. Arch Otolaryngol Head Neck Surg 2001;127:705–8.

[21] Lin CY, Lin S-L, Kao C-C, Wu J-L. The remediation of hearing deterioration in children with large vestibular aqueduct syndrome. Auris Nasus Larynx 2005;32:99–105.

[22] Furuhashi A, Sato E, Nakashima T, Miura Y, Nakayama A, Mori N, et al. Hyperbaric oxygen therapy for the treatment of large vestibular aqueduct syndrome. Undersea Hyperb Med 2001;28:195–200.

The Laryngoscope

Additional Heterozygous 2507A>C Mutation of *WFS1* in Progressive Hearing Loss at Lower Frequencies

Taro Fujikawa, MD; Yoshihiro Noguchi, MD; Taku Ito, MD; Masatoki Takahashi, MD; Ken Kitamura, MD

Objectives/Hypothesis: To describe the audiological profiles in a Japanese family with autosomal dominant hereditary sensorineural hearing loss (SNHL) and to identify the causative gene.

Study Design: A family study at an academic tertiary referral center.

Methods: A family with autosomal dominant hereditary SNHL was enrolled. Hearing loss (HL) of affected members showed mid-frequency SNHL in childhood and progressed at lower frequencies with age, resulting in low-frequency SNHL. To understand the pathology of HL of this family, we performed a genetic analysis of *WFS1*, *TECTA*, and *GJB2* by direct sequencing, and further audiovestibular examinations, including speech audiometry, distortion product otoacoustic emissions, electrocochleography, auditory brainstem responses, and electronystagmography for some affected members.

Results: A heterozygous A-to-C nucleotide transversion (c.2507A>C), resulting in a lysine-to-threonine substitution at codon 836 (K836T) was identified in exon 8 of *WFS1*. K836T was segregated with HL in the 15 participants in the genetic study but was not detected in the 212 normal chromosomes. Only polymorphic changes were detected in *TECTA* and *GJB2*. Audiovestibular examinations indicated purely cochlear HL and normal vestibular function. The summating potential/action potential ratios in electrocochleography increased in the bilateral ears of the proband.

Conclusions: The family described with autosomal dominant inheritance of K836T of the *WFS1* gene demonstrates a progressive hearing loss in the lower frequencies.

Key Words: Wolfram syndrome type 1 gene, nonsyndromic, hereditary hearing loss, DFNA6/14/38, mid-frequency, low-frequency, electrocochleography.

***Laryngoscope*, 120:166–171, 2010**

INTRODUCTION

Hearing loss (HL) is the most common form of sensory dysfunction and is caused by environmental and genetic factors. Remarkable progress has been made in mapping over 100 loci for nonsyndromic hereditary HL, and more than 40 deafness genes have been identified. Among the autosomal dominant loci (DFNA) of nonsyndromic hereditary HL, most loci are responsible for high-frequency sensorineural hearing loss (SNHL), whereas three loci (DFNA1, DFNA6/14/38, and DFNA54) are associated with low-frequency SNHL, and other loci (DFNA8/12, DFNA10, and DFNA13) are associated with mid-frequency SNHL. These characteristic features in audiometric configuration may become a powerful clue for the genetic testing of hereditary HL.

Mutations in the Wolfram syndrome type 1 gene (*WFS1*) are the cause of both the autosomal recessive condition, Wolfram syndrome, and autosomal dominant nonsyndromic HL, DFNA6/14/38. Wolfram syndrome is characterized by diabetes insipidus, juvenile onset diabetes mellitus, optic atrophy, and SNHL.[1] DFNA6/14/38 is caused by heterozygous mutations of *WFS1* and is responsible for autosomal dominant low-frequency SNHL without vestibular symptoms.[2] To date, nearly 30 different heterozygous mutations for DFNA6/14/38 have been reported, and the majority of the mutations are located in exon 8, a portion of which encodes the C-terminal domain of the wolframin protein (wolframin). Moreover, many polymorphisms of *WFS1* have been found, and some of these polymorphisms are associated with diabetes mellitus and psychiatric disorders.[1] Wolframin is an

Additional Supporting Information may be found in the online version of this article.

From the Department of Otolaryngology, Tokyo Medical and Dental University Graduate School, Tokyo, Japan.

Editor's Note: This Manuscript was accepted for publication July 21, 2009.

Presented in part at the 6th Molecular Biology of Hearing and Deafness Conference, Hinxton, United Kingdom, July 11–14, 2007.

This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 19591960, 15790924, 14370539, 16659462, 16012215) from the Ministry of Health, Labour and Welfare of Japan, and by Health and Labour Sciences Research Grants (H13-006; Researches on Sensory and Communicative Disorders, and H14-21 and No. 17242101; Research on Measures for Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan.

Send correspondence to Yoshihiro Noguchi, MD, Department of Otolaryngology, Tokyo Medical and Dental University Graduate School, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan.
E-mail: noguchi.oto@tmd.ac.jp

DOI: 10.1002/lary.20691

endoplasmic reticulum (ER) membrane protein and is widely expressed in nerve tissues, including the brain and pancreatic β cells and the inner ear.[3] Although the function of wolframin remains unknown, it is believed that wolframin is important for maintaining inner ear ion homeostasis.[3] In this study, we performed a genetic analysis in a Japanese family with autosomal dominant, nonsyndromic SNHL. In this family, affected members of the younger generation showed mid-frequency SNHL, but HL at lower frequencies progressed with age. Additional audiovestibular examinations, including electrocochleography (ECochG), were carried out to understand the pathology of the HL of this family.

MATERIALS AND METHODS

Subjects

All the procedures were approved by the Ethics Reviewing Committee of Tokyo Medical and Dental University and were carried out only after obtaining a written informed consent from each individual and/or parents of the children.

A four-generation Japanese family (TMDU342) with autosomal dominant SNHL was studied (Fig. 1A). In 15 of the 46

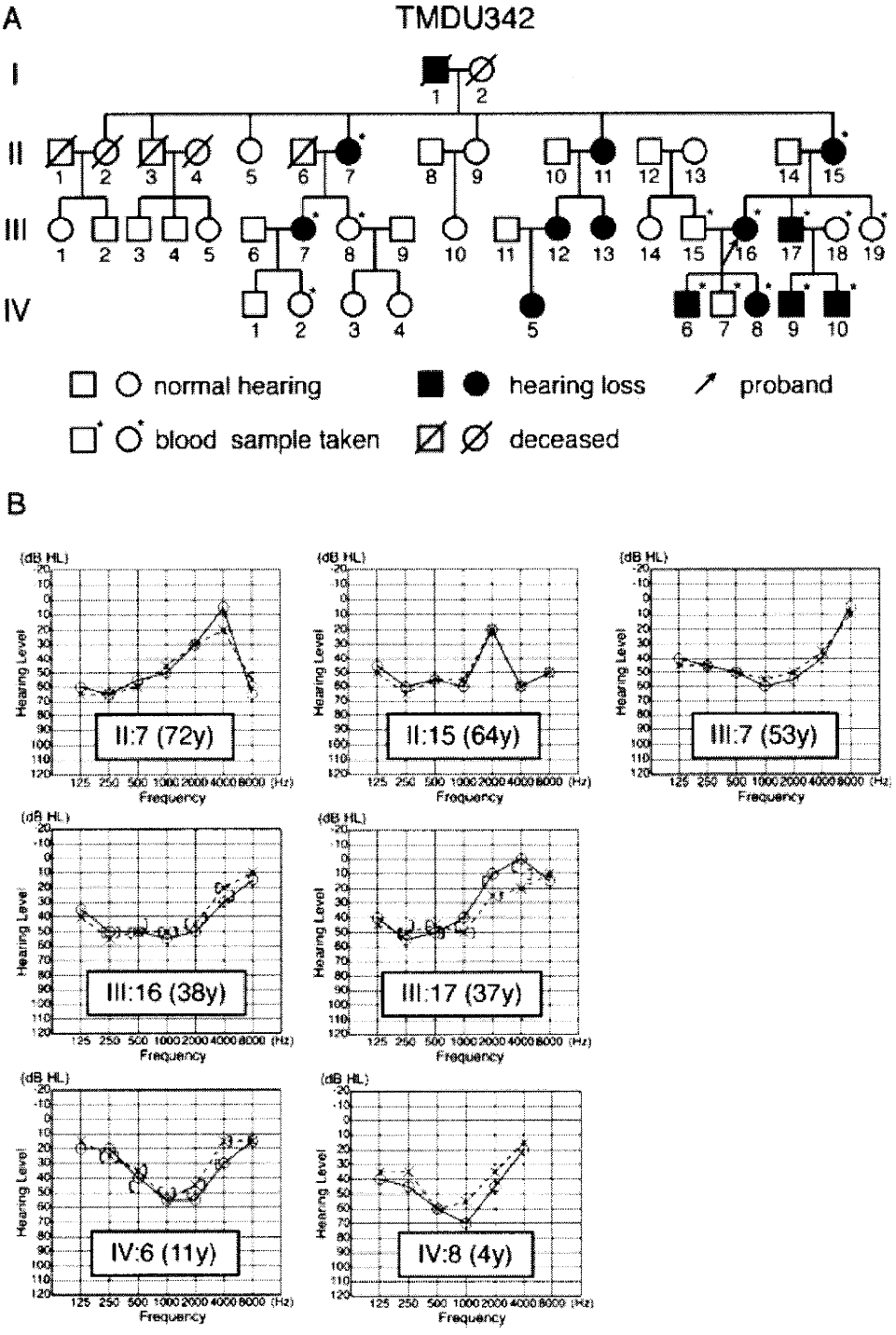

Fig. 1. (A) Pedigree of the Japanese family (TMDU342) with autosomal dominant, nonsyndromic hearing loss. (B) Audiograms in seven affected individuals. Bilateral mid-frequency hearing loss (HL) is seen in two children (IV: 6, IV: 8), whereas bilateral low-frequency hearing loss is shown in the other individuals.

family members, audiometric evaluations and a genetic analysis were performed. The information regarding HL in the other 31 family members was obtained through interviews with the proband (III: 16). Pure tone audiometry was carried out in all participants, with the exception of two young children who were 4 years (VI: 9) and 2 years (IV: 10) of age, respectively. The HL for these two children had not been found before confirming by auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Audiograms from seven affected individuals showed bilateral, symmetric SNHL with a moderate to severe degree of severity. Children (IV: 6, IV: 8) had mid-frequency SNHL, whereas middle-aged individuals (III: 7, III: 16, III: 17) had low-frequency SNHL. Audiograms from elderly individuals (II: 7, II: 15) showed low-frequency SNHL with an additional increase in audiometric thresholds at higher frequencies, which was probably due to aging (Fig. 1B). Audiograms from unaffected individuals were essentially normal. The predicted audiogram for ages ranging from 0 to 70 years (age-related typical audiogram; [ARTA]) indicated a gradual elevation of the audiometric thresholds at the lower frequencies (see Supporting Information Appendix). The onset of HL, which was obtained from taking a history, ranged from 2 to 10 years of age, but all demonstrated normal language development without the use of hearing aids. They were not aware of apparent progression of HL or vestibular symptoms. One individual (II: 7) had recognized bilateral tinnitus after receiving chemotherapy for breast cancer at 46 years of age. Affected individuals had no other abnormalities, such as diabetes insipidus, diabetes mellitus, or optic atrophy. High-resolution computed tomography of the temporal bone in three individuals (III: 16, IV: 6, IV: 8) showed no abnormal findings.

Audiovestibular Examinations

Speech audiometry, DPOAEs, and ABRs were performed in two affected individuals (III: 16, IV: 6), and tympanic ECochG was carried out in the proband (III: 16) as described previously.[4,5] In brief, in DPOAE measurement, two primary tones with a frequency ratio (f2/f1) kept at 1.22 were presented at 70 dB sound pressure level using an ILO292 analyzer (Otodynamics Ltd., Hatfield, Herts, UK). For the measurement of ABRs, alternating polarity clicks at the intensity of 90 dB nHL were presented as the acoustic stimuli at a frequency of 9.5 Hz. The inter-peak latency difference between waves I and V (IPL I-V) was used as a criterion and an IPL I-V longer than 4.4 ms was considered abnormal. In ECochG measurement, short tone-bursts with a frequency of 1 kHz were used to evoke cochlear microphonics (CM) and alternating polarity clicks were used to measure summating potentials (SP) and cochlear nerve action potentials (AP). The CM and AP detection thresholds and the SP/AP ratios at the intensity of 90 dB nHL were used as indicators.

Vestibular examinations were carried out in the proband (IV: 16). Positional, positioning, and spontaneous nystagmus tests were conducted with an infrared charge-coupled device camera. Air caloric testing was performed by electronystagmography as described previously.[5] Caloric hypoplexia was defined as maximal slow phase velocity less than 20°/s.

Genetic Analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard methods. Based on the audiometric configurations, we screened for the presence of *TECTA* and *WFS1* mutations. Exons 17, 18, 19, and 20 of *TECTA*, corresponding to the zona pellucida domain of alpha-tectorin (the product of *TECTA*), were amplified by polymerase chain reaction (PCR), because mutations in that region lead to autosomal dominant mid-frequency SNHL.[6] The entire coding regions of *WFS1* were amplified as previously described.[7] We amplified the coding region of the gap junction beta-2 gene (*GJB2*) in order to exclude the most frequent cause of nonsyndromic HL. Amplified products were purified using a QIAquick PCR purification Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions and were directly sequenced using an Applied Biosystems Prism Big Dye Terminator Cycle Sequencing Ready Kit (Applied Biosystems Inc., Foster City, CA) and an ABI Prism model 310 Genetic Analyzer (Applied Biosystems Inc.). Genetic analyses of *TECTA*, *WFS1*, and *GJB2* were initially performed in the proband. Thereafter, whenever any unreported variants causing amino acid substitutions were detected, a genetic analysis was carried out in the remaining participants and in 212 normal chromosomes.

RESULTS

Audiovestibular Findings

Table I shows audiovestibular findings of two affected individuals (III: 16, IV: 6). Speech discrimination ability was not affected in both individuals. DPOAE amplitudes were decreased to the noise level only at the mid-frequencies of f2, which corresponded to the audiometric configurations. In the proband (III: 16), the detection thresholds of the CM and AP in ECochG showed elevated values of 50 and 40 dB nHL, respectively. Interestingly, the SP/AP ratios increased to 0.41 in the right ear and to 0.51 in the left ear (see Supporting Information Appendix). The IPL I-V of ABRs was not prolonged, indicating that retrocochlear dysfunction was absent. Gaze, positional, positioning, and spontaneous nystagmus were not detected in the proband and a normal caloric response was observed for both ears.

Genetic Findings

In the proband, we detected a heterozygous A-to-C nucleotide transversion (c.2507A>C) in exon 8 of *WFS1* that was predicted to result in a lysine-to-threonine substitution at codon 836 (K836T) (see Supporting Information Appendix). Eight previously reported polymorphisms (T170T, R228R, V333I, V395V, N500N, H611R, K811K, and S855S) were also found. Additional genetic analysis showed cosegregation of K836T with HL in all participants from this family. The K836T allele was not detected in 212 normal chromosomes.

The proband had a heterozygous C-to-T transition (5634C>T) in exon 18 of *TECTA* that was predicted to result in an alanine-to-valine substitution at codon 1879. Because one more individual (III: 19) with normal hearing had the same transition, we concluded that A1879V was a polymorphic variant. The *GJB2* variants, V27I/E114G, usually found in cis, were identified in the proband.

DISCUSSION

In the present family, we detected a heterozygous K836T within the C-terminal domain of wolframin. The domain is important because most of the known

TABLE I.
Audiovestibular Findings of Two Affected Individuals.

Individual No. (Age/Sex)	Ear	SDS (%)	DP Level	ECochG			ABR (Wave I/V) (ms)	CR (MSV) (°/s)
				CM Threshold (dB nHL)	AP Threshold (dB nHL)	SP/AP		
III: 16 (38/F)	Right	95	Decreased	50	40	0.41	1.86/5.60	42
	Left	95	Decreased	50	40	0.51	1.74/5.74	38
IV: 6 (6/M)	Right	85	Decreased				1.50/5.48	
	Left	95	Decreased				1.62/5.40	

ECochG = electrocochleography; ABR = auditory brain response; CR = caloric response; SDS = speech discrimination score; DP = distortion product; CM = cochlear microphonics; nHL = normal hearing level; AP = action potentials; SP = summating potentials; MSV = maximal slow phase velocity; F = female; M = male.

mutations in DFNA6/14/38 are intensively located in this region.[1] The position (K836) is highly conserved across human (AAH30130), chicken (XP_420803), mouse (NP_035846), rat (EDM00031), and orangutan (NP_001127593), and K836T was never detected in 212 normal controls. In K836T the positively charged residue (lysine) was substituted with the noncharged, polar residue (threonine), and this substitution can most likely change the secondary structure of wolframin. Moreover, affected adult members of this family showed typical clinical features of DFNA6/14/38, low-frequency SNHL and no vestibular dysfunction. Therefore, it is thought that K836T is a novel mutation of *WFS1* and is a crucial change that allows the present family to show their clinical features.

However, affected children of the present family showed mid-frequency SNHL with normal or slightly impaired hearing at the lower frequencies. The ARTA indicated a gradual progression of the audiometric thresholds at the lower frequencies with age. Table II shows the clinical features of DFNA6/14/38 from previous reports that described audiogram.[2,7–21] HL in DFNA6/14/38 starts from birth to the early thirties and shows low-frequency SNHL with the severity of moderate to severe. In most of the families, the gradual progression in audiometric thresholds is recognized at higher frequencies. There has been no report of progression of HL at lower frequencies, as was observed in the present family. Some affected individuals in the families complained of tinnitus. None of the patients in the DFNA6/14/38 families reported either vestibular symptoms, or showed spontaneous nystagmus and/or an abnormal caloric response. Among previously reported mutations, E864K was detected in Japanese families with DFNA6/14/38,[21] as well as in a Danish family[22] with autosomal dominant optic atrophy. Some members in the Danish family had SNHL that showed various audiometric configurations, including low-frequency and flat forms, as well as U-shaped (mid-frequency) forms. Therefore, attention should be given to the fact that some heterozygous mutations in exon 8 of *WFS1* can be responsible for mid-frequency SNHL.

TABLE II.
Clinical Features of DFNA6/14/38 Families.

Amino Acid Change	Audiometric Configuration	Age at Onset	Degree of Hearing Loss (dB HL)	Progression of Hearing Loss	Tinnitus	Vestibular Examination	Reference No.
K634T	Low	<17	40–60		−		8
Y669H	Low	<22	40–60	−			9
G674E	Low	0	40–70	+		Normal	10
G674V	Low	0	40–90	+		Normal	10
R685P	Low	~4	40–90	+			11
T699M	Low	<25	40–60	+		Normal	12
K705N	Low	0	40–60	−			13
A716T	Low	<10	40–80	+	+	Normal	14,15,16
D771H	Low	5–20	40–80	−			17
L829P	Low	6–32	40–60	+	+	Normal	2,15,18
G831D	Low	<20	40–60	+	+	Normal	15,19
K836T	Middle to low	2–10	40–60	+	−	Normal	The present study
A844T	Low	<6	40–60	−	−	Normal	7
R859P	Low	5–30	40–60	−			17
R859Q	Low	2–45	40–60	+	+		20
E864K	Low	4	40–80	+			21

HL = hearing loss.