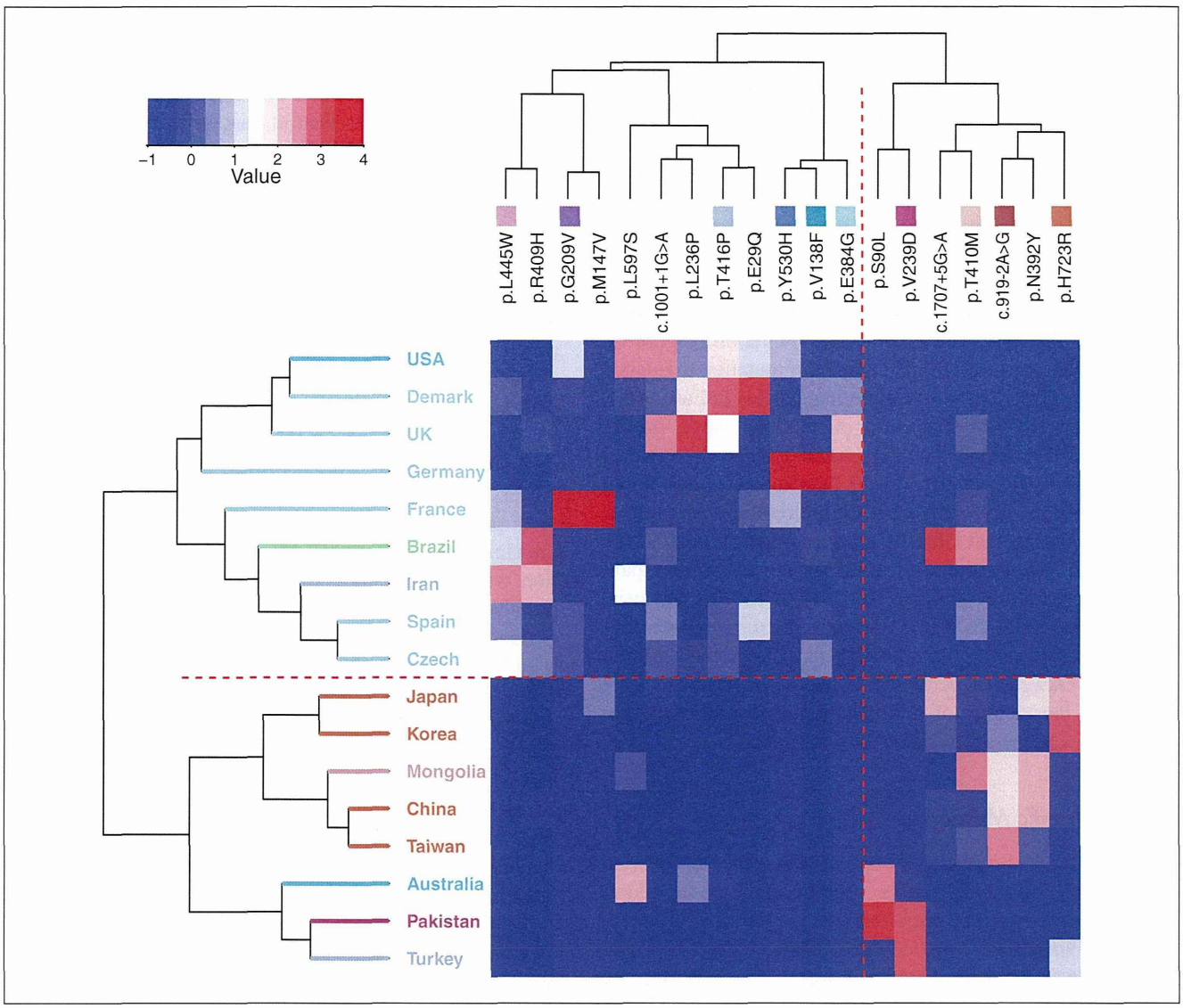

Figure 5. *SLC26A4* mutation clustering analysis.
A cluster analysis of the *SLC26A4* mutation allele frequencies shown in Table 2 and Figure 2 was performed by calculating the Euclidean distance and using Ward's clustering method to elucidate the similarities between ethnic populations.

polymorphism.[89] Later, it was described as a potential pathogenic missense mutation,[90,91] and Bruzzone et al[92] confirmed that the p.V37I mutation can impair channel activity. Although the most recurrent *GJB2* mutations exhibit severe phenotypes, the p.V37I and the p.M34T and p.L90P mutations, which are the second most prevalent mutations in some Caucasian countries, have milder phenotypes.[6] Because most studies of the *GJB2* mutation spectrum include a severe-to-profound cohort, these milder phenotype mutations may not be detected in a deaf study cohort.

Lineages associated with haplogroup DE* in the Y-haplotype tree revealed the same distribution pattern as that of p.V37I (haplogroup DE* was observed in East Asian, North African, Middle Eastern, and South European countries but not in Sub-Saharan African or South Asian countries). In those reports, haplogroup DE* was separated from other populations at an early stage of human migration (Figure 4) and was distinguished from other lineages by carrying haplogroups C and F* (which included haplogroups F-T). In our cluster analysis, Southeast Asian populations, characterized by the p.V37I mutation, were grouped inside the cluster that included East Asian, South Asian, and Sub-Saharan African populations and was distinguishable from other countries. This result also supports the notion that the genetic backgrounds of these populations were distinguishable from those of other populations.

The p.R143W Mutation

The *GJB2* p.R143W mutation is 1 of the most common causes of hearing loss in Sub-Saharan African populations (Ghana) and is observed in East Asian, European, and Middle Eastern populations at moderate to low frequencies. It is interesting that this mutation was not observed in Southeast Asian, South Asian, or North African populations. Lineages associated with haplogroup B in the Y-haplotype tree were ancestors of a Sub-Saharan population and could be associated with the origin of the p.R143W mutation; however, this lineage is restricted to Africa and was presumed to be separated at a very early stage of human migration (Figure 4). Thus, it is impossible to explain the high mutation frequency in Ghana and the low to moderate frequencies in East Asian, Middle Eastern, and European populations based on the Y-chromosome lineage. In our cluster results, the Ghanan population was clustered with the East and Southeast Asian populations and distinguishable from North African populations, suggesting that Ghanan and East and Southeast Asian populations separated during an early stage of human expansion from Africa. A possible explanation for this inconsistency in the p.R143W and Y-chromosome distributions may be the occurrence of this mutation in each of the different ancestral lineages. Nevertheless, a haplotype analysis of the region in the vicinity of this mutation in Ghana and other countries is necessary to make this conclusion.

Other Specific Mutations

The c.167*del*T mutation is found in the Ashkenazi Jewish population. Moreover, some specific pathogenic mutations occur in specific areas such as p.S99F in Colombia, c.257_259*del*CGC in Iran and Turkey, and c.313_326*del*14bp in Eastern Europe. In addition, the c.176_191*del*16bp mutation occurs in Japan and China, whereas this mutation is rare in the other regions. The p.G45E/Y136X mutation is the third most prevalent mutation in the Japanese population; however, there are no reports in other countries.

Prevalent SLC26A4 Mutations

The *SLC26A4* mutation was the second most frequent mutation in patients with NSHL. However, there have been only a limited number of studies on the frequency of the *SLC26A4* mutation performed on patients with NSHL compared to those with *GJB2* mutations. Studies on NSHL have revealed biallelic *SLC26A4* mutations in 2% to 3.5% of Caucasian patients,[17,68,93] but in 5.5% to 12.6% of East Asian patients.[58,94-96] The high prevalence (82%-97.9%) of *SLC26A4* mutations in patients with EVA is compatible with the high prevalence of *SLC26A4* mutations reported in East Asians. These frequencies are also higher than those reported in Caucasian populations (20% in the USA,[95] 40.0% in France,[64] and 27% in Spain[67]). Compared to *GJB2* mutations, there have been fewer reports on the mutation spectra and fewer mutated *SLC26A4* alleles identified in Caucasian populations than in East Asian populations.

We summarized the prevalent *SLC26A4* mutations in each ethnic population in Table 2 and Figure 2.

The p.H723R and c.919-2A>G mutations were the most common in the Asian population. p.H723R was predominant in Japan (51.0% frequency) and Korea (60.2% frequency). The frequencies of c.919-2A>G were 61.6%, 76.7%, and 62.5% in China, Taiwan, and Mongolia, respectively. p.V239D was the most frequent mutation in Turkey (33.3%) and Pakistan (35.6%). However, these mutations were not detected in the Caucasian population.

The c.1001+1G>A, p.V138F, p.T416P, p.L236P, and p.G209V mutations were prevalent in the Caucasian population. The c.1001+1G>A mutation was the most or second most prevalent mutation in 4 of 7 European countries and the United States (range, 7.1%-20.5%). The p.V138F mutation was the most prevalent in countries with Caucasian populations and was predominant in Germany (66.7%), Czechoslovakia (18.0%), and Denmark (17.3%). The p.T416P, p.L236P, and p.G209V mutations were mainly found in Denmark (20.0%), the United Kingdom (23.1%), and France (14.3%), respectively, and these mutations were found at a moderate frequency in Europe and the United States. Most of the mutations found in the Caucasian population were not found in the Asian or the Middle Eastern populations. It was evident that the *SLC26A4* mutation spectrum found in the Asian population was quite different from that in the Caucasian population.

Haplotype analyses in previous studies confirmed the founder effect of p.H723R and c.919-2A>G.[94,97] We also performed a cluster analysis of the standardized allele frequencies of the *SLC26A4* mutations to elucidate the similarities between the ethnic populations shown in Table 2 by calculating the Euclidean distance and using Ward's clustering method (Figure 5). The results of the *SLC26A4* cluster analysis were quite similar to those of the *GJB2* cluster analyses for the Japanese, Korean, Chinese, Taiwanese, and Mongolian populations, which are characterized by the p.H723R, p.N392Y, c.919-2A>G, p.T410M, and c.1707+5G>A mutations, and were grouped into 1 cluster. This result clearly indicates the similarities in the genetic backgrounds between the East Asian population and the *GJB2* c.235*del*C distribution. The *GJB2* mutation analysis results indicate that haplogroup NO* may be an ancestor of these mutations. In contrast, most of the European populations were grouped into 1 large cluster (Figure 5). It is interesting that the European populations were divided into 2 clusters at the bottom of the clustering tree. One cluster included Denmark, the United Kingdom, the United States, and Germany, whose populations were characterized by

p.E29Q, p.T416P, p.L236P, c.1001+G>A, and p.L597S mutations, and the other included the French, Spanish, Czech, and Iranian populations, characterized by the p.G209V, p.R409H, and p.L445W mutations. This distribution pattern was quite similar to the Y-chromosome haplotype distribution for modern European populations. The ancestor of the northern part of Europe (including Danish, British, and German populations) was presumed to be haplogroup R and that for the southern part of Europe (including many Mediterranean countries) was presumed to be haplotype I. This observation could be the reason for the differences among the 2 groups of European populations.

The p.V239D mutation was the most common mutation in Pakistan and Turkey. It is unfortunate that no reports have described *SLC26A4* mutations in Africa, the roots of humans; thus, future studies are required to define the origin of the mutation and the *SLC26A4* mutation distributions worldwide.

GJB2 *and* SLC26A4 *Mutation Origins*

In this review article, we summarized the 2 major causes of hearing loss, the *GJB2* and *SLC26A4* gene mutation spectra, in many ethnic populations and also performed clustering analysis for the *GJB2* and *SLC26A4* gene mutations. We also performed a comparative analysis between the clustering analysis results and the Y-chromosomal haplogroup analysis results, which revealed human migration routes.

The combination of the results for *GJB2* and *SLC26A4* shows that many mutation distributions are well explained by founder effects in ancient human lineages, as predicted from Y-chromosome haplotype analysis. p.R143W and p.V37I mutations in *GJB2* are spread widely across the globe and are speculated to have occurred at a very early stage in human migration and have been passed down to descendants for a very long time. The p.T410M mutation in *SLC26A4* is also observed in many ethnic populations and may also have occurred at a very early stage in human migration.

Most of the common mutations, such as c.35*del*G, c.235*del*C, and p.W24X of the *GJB2* gene and p.H723R, c.919-2A>G, p.V239D, p.V138F, p.T416P, p.L236P, and p.L445W, are clearly separated into 2 large subgroups: 1 includes the c.235*del*C and p.W24X mutations of *GJB2* and the p.H723R, c.919-2A>G, and p.V239D mutations of *SLC26A4* observed in the East Asian, South Asian, and Southeast Asian populations, whereas the other includes the c.35*del*G mutation of *GJB2* and the p.V138F, p.T416P, p.L236P, and p.L445W mutations of *SLC26A4* observed in the North African, European, Middle Eastern, and North Eurasian populations. This disequilibrium in the distribution of these mutations reveals that these gene mutations occurred after the branching off of each ancestral lineage. It is interesting that many previous reports proposed the origin of these mutations to be in the Middle East or the southern part of Central Asia, areas proposed to contain the roots of many populations belonging to haplogroup F* to T of the Y-chromosome haplogroup.

On the other hand, there are a number of very restricted mutations such as *GJB2*: p.S99F in the Colombian, c.257_259*del*CGC in the Iranian and Turkish, c.313_326*del*14bp in the Eastern European, c.176_191*del*16bp in the Japanese and Chinese, and p.G45E/Y136X in the Japanese populations, as well as many *SLC26A4* mutations. This restricted distribution of these mutations might reflect the fact that these mutations occurred more recently in our ancestors after migration. Haplotype analysis of the region in the vicinity of these mutations is necessary to confirm this conclusion.

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References

1. Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci*. 1991;630:16-31.
2. Azaiez H, Chamberlin GP, Fischer SM, et al. *GJB2*: the spectrum of deafness-causing allele variants and their phenotype. *Hum Mutat*. 2004;24:305-311.
3. Dai P, Yu F, Han B, et al. *GJB2* mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. *J Transl Med*. 2009;7:26.
4. Tsukada K, Nishio S, Usami S; Deafness Gene Study Consortium. A large cohort study of *GJB2* mutations in Japanese hearing loss patients. *Clin Genet*. 2010;78:464-470.
5. Chan DK, Chang KW. *GJB2*-associated hearing loss: systematic review of worldwide prevalence, genotype, and auditory phenotype. *Laryngoscope*. 2014;124:E34-E53.
6. Snoeckx RL, Huygen PL, Feldmann D, et al. *GJB2* mutations and degree of hearing loss: a multicenter study. *Am J Hum Genet*. 2005;77:945-957.
7. Miyagawa M, Nishio SY, Usami S; Deafness Gene Study Consortium. Mutation spectrum and genotype-phenotype correlation of hearing loss patients caused by *SLC26A4* mutations in the Japanese: a large cohort study. *J Hum Genet*. 2014;59:262-268.
8. Shin JW, Lee SC, Lee HK, Park HJ. Genetic screening of *GJB2* and *SLC26A4* in Korean cochlear implantees:

experience of Soree Ear Clinic. *Clin Exp Otorhinolaryngol.* 2012;5(suppl 1):S10-S13.
9. Wu CC, Hung CC, Lin SY, et al. Newborn genetic screening for hearing impairment: a preliminary study at a tertiary center. *PLoS One.* 2011;6:e22314.
10. Tekin M, Xia XJ, Erdenetungalag R, et al. *GJB2* mutations in Mongolia: complex alleles, low frequency, and reduced fitness of the deaf. *Ann Hum Genet.* 2010;74:155-164.
11. Snoeckx RL, Djelantik B, Van Laer L, Van de Heyning P, Van Camp G. *GJB2* (connexin 26) mutations are not a major cause of hearing loss in the Indonesian population. *Am J Med Genet A.* 2005;135:126-129.
12. Zainal SA, Md Daud MK, Abd Rahman N, Zainuddin Z, Alwi Z. Mutation detection in *GJB2* gene among Malays with non-syndromic hearing loss. *Int J Pediatr Otorhinolaryngol.* 2012;76:1175-1179.
13. Wattanasirichaigoon D, Limwongse C, Jariengprasert C, et al. High prevalence of V37I genetic variant in the connexin-26 (*GJB2*) gene among non-syndromic hearing-impaired and control Thai individuals. *Clin Genet.* 2004;66:452-460.
14. Padma G, Ramchander PV, Nandur UV, Padma T. *GJB2* and *GJB6* gene mutations found in Indian probands with congenital hearing impairment. *J Genet.* 2009;88:267-272.
15. Santos RL, Wajid M, Pham TL, et al. Low prevalence of connexin 26 (*GJB2*) variants in Pakistani families with autosomal recessive non-syndromic hearing impairment. *Clin Genet.* 2005;67:61-68.
16. Bajaj Y, Sirimanna T, Albert DM, Qadir P, Jenkins L, Bitner-Glindzicz M. Spectrum of *GJB2* mutations causing deafness in the British Bangladeshi population. *Clin Otolaryngol.* 2008;33:313-318.
17. Dahl HH, Ching TY, Hutchison W, Hou S, Seeto M, Sjahalam-King J. Etiology and audiological outcomes at 3 years for 364 children in Australia. *PLoS One.* 2013;8:e59624.
18. Bazazzadegan N, Nikzat N, Fattahi Z, et al. The spectrum of *GJB2* mutations in the Iranian population with non-syndromic hearing loss—a twelve year study. *Int J Pediatr Otorhinolaryngol.* 2012;76:1164-1174.
19. Brownstein Z, Avraham KB. Deafness genes in Israel: implications for diagnostics in the clinic. *Pediatr Res.* 2009;66:128-134.
20. Tekin M, Arici ZS. Genetic epidemiological studies of congenital/prelingual deafness in Turkey: population structure and mating type are major determinants of mutation identification. *Am J Med Genet A.* 2007;143A:1583-1591.
21. Imtiaz F, Taibah K, Ramzan K, et al. A comprehensive introduction to the genetic basis of non-syndromic hearing loss in the Saudi Arabian population. *BMC Med Genet.* 2011;12:91.
22. Al-Sebeih K, Al-Kandari M, Al-Awadi SA, et al. Connexin 26 gene mutations in non-syndromic hearing loss among Kuwaiti patients. *Med Princ Pract.* 2014;23:74-79.
23. Al-Achkar W, Moassass F, Al-Halabi B, Al-Ablog A. Mutations of the connexin 26 gene in families with non-syndromic hearing loss. *Mol Med Rep.* 2011;4:331-335.
24. Posukh O, Pallares-Ruiz N, Tadinova V, Osipova L, Claustres M, Roux AF. First molecular screening of deafness in the Altai Republic population. *BMC Med Genet.* 2005;6:12.
25. Minarik G, Tretinarova D, Szemes T, Kadasi L. Prevalence of DFNB1 mutations in Slovak patients with non-syndromic hearing loss. *Int J Pediatr Otorhinolaryngol.* 2012;76:400-403.
26. Danilenko N, Merkulava E, Siniauskaya M, et al. Spectrum of genetic changes in patients with non-syndromic hearing impairment and extremely high carrier frequency of 35*del*G *GJB2* mutation in Belarus. *PLoS One.* 2012;7:e36354.
27. Teek R, Kruustuk K, Zordania R, et al. Prevalence of c.35*del*G and p.M34T mutations in the *GJB2* gene in Estonia. *Int J Pediatr Otorhinolaryngol.* 2010;74:1007-1012.
28. Wiszniewski W, Sobieszczanska-Radoszewska L, Nowakowska-Szyrwinska E, Obersztyn E, Bal J. High frequency of *GJB2* gene mutations in Polish patients with prelingual nonsyndromic deafness. *Genet Test.* 2001;5:147-148.
29. Sansović I, Knezević J, Musani V, Seeman P, Barisić I, Pavelić J. *GJB2* mutations in patients with nonsyndromic hearing loss from Croatia. *Genet Test Mol Biomarkers.* 2009;13:693-699.
30. Seeman P, Malikova M, Raskova D, et al. Spectrum and frequencies of mutations in the *GJB2* (Cx26) gene among 156 Czech patients with pre-lingual deafness. *Clin Genet.* 2004;66:152-157.
31. Popova DP, Kaneva R, Varbanova S, Popov TM. Prevalence of GBJ2 mutations in patients with severe to profound congenital nonsyndromic sensorineural hearing loss in Bulgarian population. *Eur Arch Otorhinolaryngol.* 2012;269:1589-1592.
32. Radulescu L, Martu C, Birkenhager R, Cozma S, Ungureanu L, Laszig R. Prevalence of mutations located at the dfnb1 locus in a population of cochlear implanted children in eastern Romania. *Int J Pediatr Otorhinolaryngol.* 2012;76:90-94.
33. Janecke AR, Hirst-Stadlmann A, Gunther B, et al. Progressive hearing loss, and recurrent sudden sensorineural hearing loss associated with *GJB2* mutations—phenotypic spectrum and frequencies of *GJB2* mutations in Austria. *Hum Genet.* 2002;111:145-153.
34. Santos RL, Aulchenko YS, Huygen PL, et al. Hearing impairment in Dutch patients with connexin 26 (*GJB2*) and connexin 30 (GJB6) mutations. *Int J Pediatr Otorhinolaryngol.* 2005;69:165-174.
35. Marlin S, Feldmann D, Blons H, et al. *GJB2* and GJB6 mutations: genotypic and phenotypic correlations in a large cohort of hearing-impaired patients. *Arch Otolaryngol Head Neck Surg.* 2005;131:481-487.
36. Zoll B, Petersen L, Lange K, et al. Evaluation of Cx26/*GJB2* in German hearing impaired persons: mutation spectrum and detection of disequilibrium between M34T (c.101T>C) and -493*del*10. *Hum Mutat.* 2003;21:98.
37. D'Andrea P, Veronesi V, Bicego M, et al. Hearing loss: frequency and functional studies of the most common connexin26 alleles. *Biochem Biophys Res Commun.* 2002;296:685-691.
38. Rabionet R, Zelante L, Lopez-Bigas N, et al. Molecular basis of childhood deafness resulting from mutations in the *GJB2* (connexin 26) gene. *Hum Genet.* 2000;106:40-44.
39. Pampanos A, Economides J, Iliadou V, et al. Prevalence of *GJB2* mutations in prelingual deafness in the Greek population. *Int J Pediatr Otorhinolaryngol.* 2002;65:101-108.
40. Nogueira C, Coutinho M, Pereira C, Tessa A, Santorelli FM, Vilarinho L. Molecular investigation of pediatric Portuguese patients with sensorineural hearing loss [published online September 25, 2011]. *Genet Res Int.* doi:10.4061/2011/587602.
41. Carlsson PI, Karltorp E, Carlsson-Hansen E, Ahlman H, Moller C, Vondobeln U. *GJB2* (connexin 26) gene mutations among hearing-impaired persons in a Swedish cohort. *Acta Otolaryngol.* 2012;132:1301-1305.

42. Löppönen T, Väisänen ML, Luotonen M, et al. Connexin 26 mutations and nonsyndromic hearing impairment in northern Finland. *Laryngoscope*. 2003;113:1758-1763.
43. Siem G, Fagerheim T, Jonsrud C, et al. Causes of hearing impairment in the Norwegian paediatric cochlear implant program. *Int J Audiol*. 2010;49:596-605.
44. Homøe P, Koch A, Rendtorff ND, et al. *GJB2* (connexin-26) mutations are not frequent among hearing impaired patients in east Greenland. *Int J Audiol*. 2012;51:433-436.
45. Putcha GV, Bejjani BA, Bleoo S, et al. A multicenter study of the frequency and distribution of *GJB2* and GJB6 mutations in a large North American cohort. *Genet Med*. 2007;9:413-426.
46. de la Luz Arenas-Sordo M, Menendez I, Hernandez-Zamora E, et al. Unique spectrum of *GJB2* mutations in Mexico. *Int J Pediatr Otorhinolaryngol*. 2012;76:1678-1680.
47. Batissoco AC, Abreu-Silva RS, Braga MC, et al. Prevalence of *GJB2* (connexin-26) and GJB6 (connexin-30) mutations in a cohort of 300 Brazilian hearing-impaired individuals: implications for diagnosis and genetic counseling. *Ear Hear*. 2009;30:1-7.
48. Tamayo ML, Olarte M, Gelvez N, et al. Molecular studies in the *GJB2* gene (Cx26) among a deaf population from Bogota, Colombia: results of a screening program. *Int J Pediatr Otorhinolaryngol*. 2009;73:97-101.
49. Gravina LP, Foncuberta ME, Prieto ME, Garrido J, Barreiro C, Chertkoff L. Prevalence of DFNB1 mutations in Argentinean children with non-syndromic deafness. Report of a novel mutation in *GJB2*. *Int J Pediatr Otorhinolaryngol*. 2010;74:250-254.
50. Cifuentes L, Arancibia M, Torrente M, Acuna M, Farfan C, Rios C. Prevalence of the 35*del*G mutation in the *GJB2* gene in two samples of non-syndromic deaf subjects from Chile. *Biol Res*. 2013;46:239-242.
51. Snoeckx RL, Hassan DM, Kamal NM, Van Den Bogaert K, Van Camp G. Mutation analysis of the *GJB2* (connexin 26) gene in Egypt. *Hum Mutat*. 2005;26:60-61.
52. Ammar-Khodja F, Faugere V, Baux D, et al. Molecular screening of deafness in Algeria: high genetic heterogeneity involving DFNB1 and the Usher loci, DFNB2/USH1B, DFNB12/USH1D and DFNB23/USH1F. *Eur J Med Genet*. 2009;52:174-179.
53. Abidi O, Boulouiz R, Nahili H, et al. *GJB2* (connexin 26) gene mutations in Moroccan patients with autosomal recessive non-syndromic hearing loss and carrier frequency of the common *GJB2*-35*del*G mutation. *Int J Pediatr Otorhinolaryngol*. 2007;71:1239-1245.
54. Gasmelseed NM, Schmidt M, Magzoub MM, et al. Low frequency of deafness-associated *GJB2* variants in Kenya and Sudan and novel *GJB2* variants. *Hum Mutat*. 2004;23: 206-207.
55. Trabelsi M, Bahri W, Habibi M, et al. *GJB2* and GJB6 screening in Tunisian patients with autosomal recessive deafness. *Int J Pediatr Otorhinolaryngol*. 2013;77:714-716.
56. Javidnia H, Carson N, Awubwa M, Byaruhanga R, Mack D, Vaccani JP. Connexin gene mutations among Ugandan patients with nonsyndromic sensorineural hearing loss. *Laryngoscope*. 2014;124:E373-E376.
57. Hamelmann C, Amedofu GK, Albrecht K, et al. Pattern of connexin 26 (*GJB2*) mutations causing sensorineural hearing impairment in Ghana. *Hum Mutat*. 2001;18:84-85.
58. Yuan Y, Guo W, Tang J, et al. Molecular epidemiology and functional assessment of novel allelic variants of *SLC26A4* in non-syndromic hearing loss patients with enlarged vestibular aqueduct in China. *PLoS One*. 2012;7:e49984.
59. Wu CC, Lu YC, Chen PJ, et al. Phenotypic analyses and mutation screening of the *SLC26A4* and FOXI1 genes in 101 Taiwanese families with bilateral nonsyndromic enlarged vestibular aqueduct (DFNB4) or Pendred syndrome. *Audiol Neurootol*. 2010;15:57-66.
60. Dai P, Yuan Y, Huang D, et al. Molecular etiology of hearing impairment in Inner Mongolia: mutations in *SLC26A4* gene and relevant phenotype analysis. *J Transl Med*. 2008;6:74.
61. Anwar S, Riazuddin S, Ahmed ZM, et al. *SLC26A4* mutation spectrum associated with DFNB4 deafness and Pendred's syndrome in Pakistanis. *J Hum Genet*. 2009;54:266-270.
62. Kahrizi K, Mohseni M, Nishimura C, et al. Identification of *SLC26A4* gene mutations in Iranian families with hereditary hearing impairment. *Eur J Pediatr*. 2009;168:651-653.
63. Tekin M, Akcayoz D, Comak E, et al. Screening the *SLC26A4* gene in probands with deafness and goiter (Pendred syndrome) ascertained from a large group of students of the schools for the deaf in Turkey. *Clin Genet*. 2003;64:371-374.
64. Albert S, Blons H, Jonard L, et al. *SLC26A4* gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. *Eur J Hum Genet*. 2006;14:773-779.
65. Borck G, Roth C, Martine U, Wildhardt G, Pohlenz J. Mutations in the PDS gene in German families with Pendred's syndrome: V138F is a founder mutation. *J Clin Endocrinol Metab*. 2003;88:2916-2921.
66. Coyle B, Reardon W, Herbrick JA, et al. Molecular analysis of the PDS gene in Pendred syndrome. *Hum Mol Genet*. 1998;7:1105-1112.
67. Pera A, Villamar M, Vinuela A, et al. A mutational analysis of the *SLC26A4* gene in Spanish hearing-impaired families provides new insights into the genetic causes of Pendred syndrome and DFNB4 hearing loss. *Eur J Hum Genet*. 2008;16:888-896.
68. Pourova R, Janousek P, Jurovcik M, et al. Spectrum and frequency of *SLC26A4* mutations among Czech patients with early hearing loss with and without enlarged vestibular aqueduct (EVA). *Ann Hum Genet*. 2010;74:299-307.
69. Rendtorff ND, Schrijver I, Lodahl M, et al. *SLC26A4* mutation frequency and spectrum in 109 Danish Pendred syndrome/DFNB4 probands and a report of nine novel mutations. *Clin Genet*. 2013;84:388-391.
70. Campbell C, Cucci RA, Prasad S, et al. Pendred syndrome, DFNB4, and PDS/*SLC26A4* identification of eight novel mutations and possible genotype-phenotype correlations. *Hum Mutat*. 2001;17:403-411.
71. de Moraes VC, dos Santos NZ, Ramos PZ, Svidnicki MC, Castilho AM, Sartorato EL. Molecular analysis of *SLC26A4* gene in patients with nonsyndromic hearing loss and EVA: identification of two novel mutations in Brazilian patients. *Int J Pediatr Otorhinolaryngol*. 2013;77:410-413.

72. Y Chromosome Consortium. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res*. 2002;12:339-348.
73. Jobling MA, Tyler-Smith C. The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet*. 2003;4:598-612.
74. Karafet TM, Mendez FL, Meilerman MB, Underhill PA, Zegura SL, Hammer MF. New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. *Genome Res*. 2008;18:830-838.
75. Van Laer L, Coucke P, Mueller RF, et al. A common founder for the 35*del*G *GJB2* gene mutation in connexin 26 hearing impairment. *J Med Genet*. 2001;38:515-518.
76. Abidi O, Boulouiz R, Nahili H, et al. The analysis of three markers flanking *GJB2* gene suggests a single origin of the most common 35delG mutation in the Moroccan population. *Biochem Biophys Res Commun*. 2008;377:971-974.
77. Kokotas H, Van Laer L, Grigoriadou M, et al. Strong linkage disequilibrium for the frequent *GJB2* 35delG mutation in the Greek population. *Am J Med Genet A*. 2008;146A:2879-2884.
78. Najmabadi H, Nishimura C, Kahrizi K, et al. *GJB2* mutations: passage through Iran. *Am J Med Genet A*. 2005;133A:132-137.
79. Dzhemileva LU, Posukh OL, Barashkov NA, et al. Haplotype diversity and reconstruction of ancestral haplotype associated with the c.35*del*G mutation in the *GJB2* (Cx26) gene among the Volgo-Ural populations of Russia. *Acta Naturae*. 2011;3:52-63.
80. Yan D, Park HJ, Ouyang XM, et al. Evidence of a founder effect for the 235*del*C mutation of *GJB2* (connexin 26) in east Asians. *Hum Genet*. 2003;114:44-50.
81. 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;112:329-333.
82. Dzhemileva LU, Barashkov NA, Posukh OL, et al. Carrier frequency of *GJB2* gene mutations c.35*del*G, c.235*del*C and c.167*del*T among the populations of Eurasia. *J Hum Genet*. 2010;55:749-754.
83. Yao J, Lu Y, Wei Q, Cao X, Xing G. A systematic review and meta-analysis of 235*del*C mutation of *GJB2* gene. *J Transl Med*. 2012;10:136.
84. Barashkov NA, Dzhemileva LU, Fedorova SA, et al. Carrier frequency of *GJB2* gene mutations c.35*del*G, c.235*del*C and c.167*del*T among the populations of Eurasia. *J Hum Genet*. 2010;55:749-754.
85. Seeman P, Sakmaryova I. High prevalence of the IVS 1 + 1 G to A/*GJB2* mutation among Czech hearing impaired patients with monoallelic mutation in the coding region of *GJB2*. *Clin Genet*. 2006;69:410-413.
86. RamShankar M, Girirajan S, Dagan O, et al. Contribution of connexin26 (*GJB2*) mutations and founder effect to non-syndromic hearing loss in India. *J Med Genet*. 2003;40:e68.
87. Joseph AY, Rasool TJ. High frequency of connexin26 (*GJB2*) mutations associated with nonsyndromic hearing loss in the population of Kerala, India. *Int J Pediatr Otorhinolaryngol*. 2009;73:437-443.
88. Han SH, Park HJ, Kang EJ, et al. Carrier frequency of *GJB2* (connexin-26) mutations causing inherited deafness in the Korean population. *J Hum Genet*. 2008;53:1022-1028.
89. Kelley PM, Harris DJ, Comer BC, et al. Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNB1) hearing loss. *Am J Hum Genet*. 1998;62:792-799.
90. Bason L, Dudley T, Lewis K, et al. Homozygosity for the V37I connexin 26 mutation in three unrelated children with sensorineural hearing loss. *Clin Genet*. 2002;61:459-464.
91. Wilcox SA, Saunders K, Osborn AH, et al. High frequency hearing loss correlated with mutations in the *GJB2* gene. *Hum Genet*. 2000;106:399-405.
92. Bruzzone R, Veronesi V, Gomes D, et al. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. *FEBS Lett*. 2003;533:79-88.
93. Hutchin T, Coy NN, Conlon H, et al. Assessment of the genetic causes of recessive childhood non-syndromic deafness in the UK—implications for genetic testing. *Clin Genet*. 2005;68:506-512.
94. Park HJ, Shaukat S, Liu XZ, 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;40:242-248.
95. Dai P, Stewart AK, Chebib F, et al. Distinct and novel *SLC26A4*/Pendrin mutations in Chinese and U.S. patients with nonsyndromic hearing loss. *Physiol Genomics*. 2009;38:281-290.
96. Guo YF, Liu XW, Guan J, et al. *GJB2, SLC26A4* and mitochondrial DNA A1555G mutations in prelingual deafness in Northern Chinese subjects. *Acta Otolaryngol*. 2008;128:297-303.
97. Wu CC, Yeh TH, Chen PJ, Hsu CJ. Prevalent *SLC26A4* mutations in patients with enlarged vestibular aqueduct and/or Mondini dysplasia: a unique spectrum of mutations in Taiwan, including a frequent founder mutation. *Laryngoscope*. 2005;115:1060-1064.

Identification of a Novel *CLRN1* Gene Mutation in Usher Syndrome Type 3: Two Case Reports

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Abstract

Objective: This study examines the *CLRN1* gene mutation analysis in Japanese patients who were diagnosed with Usher syndrome type 3 (USH3) on the basis of clinical findings.

Methods: Genetic analysis using massively parallel DNA sequencing (MPS) was conducted to search for 9 causative USH genes in 2 USH3 patients.

Results: We identified the novel pathogenic mutation in the *CLRN1* gene in 2 patients. The missense mutation was confirmed by functional prediction software and segregation analysis. Both patients were diagnosed as having USH3 caused by the *CLRN1* gene mutation.

Conclusion: This is the first report of USH3 with a *CLRN1* gene mutation in Asian populations. Validating the presence of clinical findings is imperative for properly differentiating among USH subtypes. In addition, mutation screening using MPS enables the identification of causative mutations in USH. The clinical diagnosis of this phenotypically variable disease can then be confirmed.

Keywords

CLRN1, Usher syndrome, deaf-blindness, massively parallel sequencing, genetics of hearing loss

Introduction

Usher syndrome (USH) is an autosomal recessive disorder characterized by hearing loss (HL), retinitis pigmentosa (RP), and vestibular dysfunction. Three clinical subtypes of USH can be distinguished, among which USH type 1 (USH1) is the most severe because of profound HL, absent vestibular response, and prepubertal onset RP. USH type 2 (USH2) is characterized by congenital moderate to severe HL with a high-frequency sloping configuration. Vestibular function is normal and onset of RP is in the first or second decades of life. USH type 3 (USH3) is characterized by the variable onsets of progressive HL and RP and the variable impairment of vestibular function (normal to absent).[1] However, differential diagnosis between clinical subtypes is sometimes difficult when clinical findings are limited.

To date, 10 corresponding genes have been identified as a cause of USH: *MYO7A, USH1C, CDH23, PCDH15, USH1G*, and *CIB2* for USH1 and *USH2A, GPR98*, and *DFNB31* for USH2. For USH3, only *CLRN1* has been described (Hereditary Hearing loss Homepage; http://hereditaryhearingloss.org). However, the size of these targeted genes is large, and much labor and expense are required for analysis when using conventional Sanger sequencing. Recent advances in targeted genomic enrichment with massively parallel sequencing (TGE+MPS) have made it possible to sequence all known causative genes simultaneously.[2,3] We previously reported USH mutation analysis using MPS and the frequency of USH1 genes in Japanese USH1 patients.[4]

In this study, we have conducted genetic analysis using TGE+MPS technology to search for 9 causative USH genes (excluding *CIB2*) in Japanese USH patients. Here, we report that 2 patients could be clinically diagnosed as having USH3, and that *CLRN1* gene mutation analysis confirmed

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the clinical diagnosis. This is the first report of USH3 caused by *CLRN1* mutation in an Asian population, and highlights the importance of causative mutation analysis for the confirmation of the clinical diagnosis in USH3 patients.

Materials and Methods

Subjects

Case 1 (Figure 1). The patient (III-3; KYS5003), a Japanese 58-year-old woman, was the third child of healthy consanguineous parents. She had 2 healthy brothers (Figure 1A). She experienced night blindness at age 8 and suffered from constricted vision in junior high school. She was diagnosed as having RP at that time. She later suffered from light perception vision (right) and hand motion vision (left) at age 58. She became aware of HL at age 40, which was diagnosed as mild HL at age 50 (Figure 1B). She began to wear hearing aids from age 50. Her HL did not deteriorate over a period of 8 years from age 50 to 58 (Figure 1C). She has never experienced vertigo. Caloric tests showed normal responses.

Case 2 (Figure 2). The patient (IV-7; SGA5001), a Japanese 61-year-old man, was the fourth child of healthy consanguineous parents. He had 3 healthy brothers (Figure 2A). He experienced night blindness and was diagnosed as having RP at age 30. He suffered from light perception vision at age 61. He became aware of HL at age 40. He began to wear hearing aids from age 52, and was diagnosed as severe HL at age 56 (Figure 2B). His pronunciation was clear and his speech was completely intelligible. His HL did not deteriorate over a period of 5 years from age 56 to 61 (Figure 2C). He has never experienced vertigo. Caloric tests showed normal responses.

Based on the above findings, we considered the diagnosis of the patients to be USH. It was highly likely that the clinical subtype was USH3, because the HL of both patients was late-onset.

Subsequently, to confirm the clinical diagnosis, mutation analysis of reported USH genes was performed. All subjects (or guardians on the behalf of minors) gave prior written informed consent for participation in the study, which was approved by the Ethical Committee of Shinshu University.

Massively Parallel Sequencing

Targeted Genes

MYO7A, [NM_000260.3]; *USH1C*, [NM_153676.3]; *CDH23*, [NM_022124.5]; *PCDH15*, [NM_033056.3]; *USH1G*, [NM_173477.2]; *USH2A*, [NM_206933.2]; *GPR98*, [NM_032119.3]; *DFNB31*, [NM_015404.3]; *CLRN1*, [NM_174878.2].

Amplicon Library Preparation

Amplicon libraries were prepared using an Ion AmpliSeq™ Custom Panel (Life Technologies, Foster City, CA, USA) for 9 USH genes according to the manufacturer's instructions. The detailed protocol is described elsewhere.[5] After preparation, the amplicon libraries were diluted to 20pM, and equal amounts of 2 libraries for 2 patients were pooled for 1 sequence reaction.

Emulsion PCR and Sequencing

Emulsion PCR and sequencing were performed according to the manufacturer's instructions. The detailed protocol is described elsewhere.[5] MPS was performed with an Ion Torrent Personal Genome Machine (PGM) system using an Ion PGM™ 200 Sequencing Kit and an Ion 318™ Chip (Life Technologies).

Base Call and Data Analysis

Sequence data were mapped against the human genome sequence (build GRCh37/hg19) with a Torrent Mapping Alignment Program. After sequence mapping, DNA variant regions were compiled with Torrent Variant Caller plug-in software. After variant detection, their effects were analyzed using ANNOVAR software.[6,7] The missense, nonsense, insertion/deletion, and splicing variants were then selected from among the identified variants. Variants were further selected as less than 1% of: (1) the 1000 genome database (http://www.1000genomes.org/), (2) the 6500 exome variants (http://evs.gs.washington.edu/EVS/), (3) the Human Genetic Variation Database (data set for 1208 Japanese exome variants) (http://www.genome.med.kyoti-u.ac.jp/SnpDB/index.html), and (4) the 188 in-house Japanese normal hearing controls.

To predict the pathogenicity of missense variants, the following functional prediction software was used: Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/), Polymorphism Phenotyping (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/), LRT (http://www.genetics.wustl.edu/jflab/lrt_query.html), and MutationTaster (http://www.mutationtaster.org/). Candidate mutations were confirmed by Sanger sequencing, and the responsible mutations were identified by segregation analysis using samples from among the patients' family members.

Results

Mutation analysis of the 9 USH genes in both probands revealed the same probable pathogenic mutation in *CLRN1*, c.[606T>G] (p.[N202K]), which was a mutation not detected in 188 control subjects with normal hearing. This residue is well conserved among several species. We also

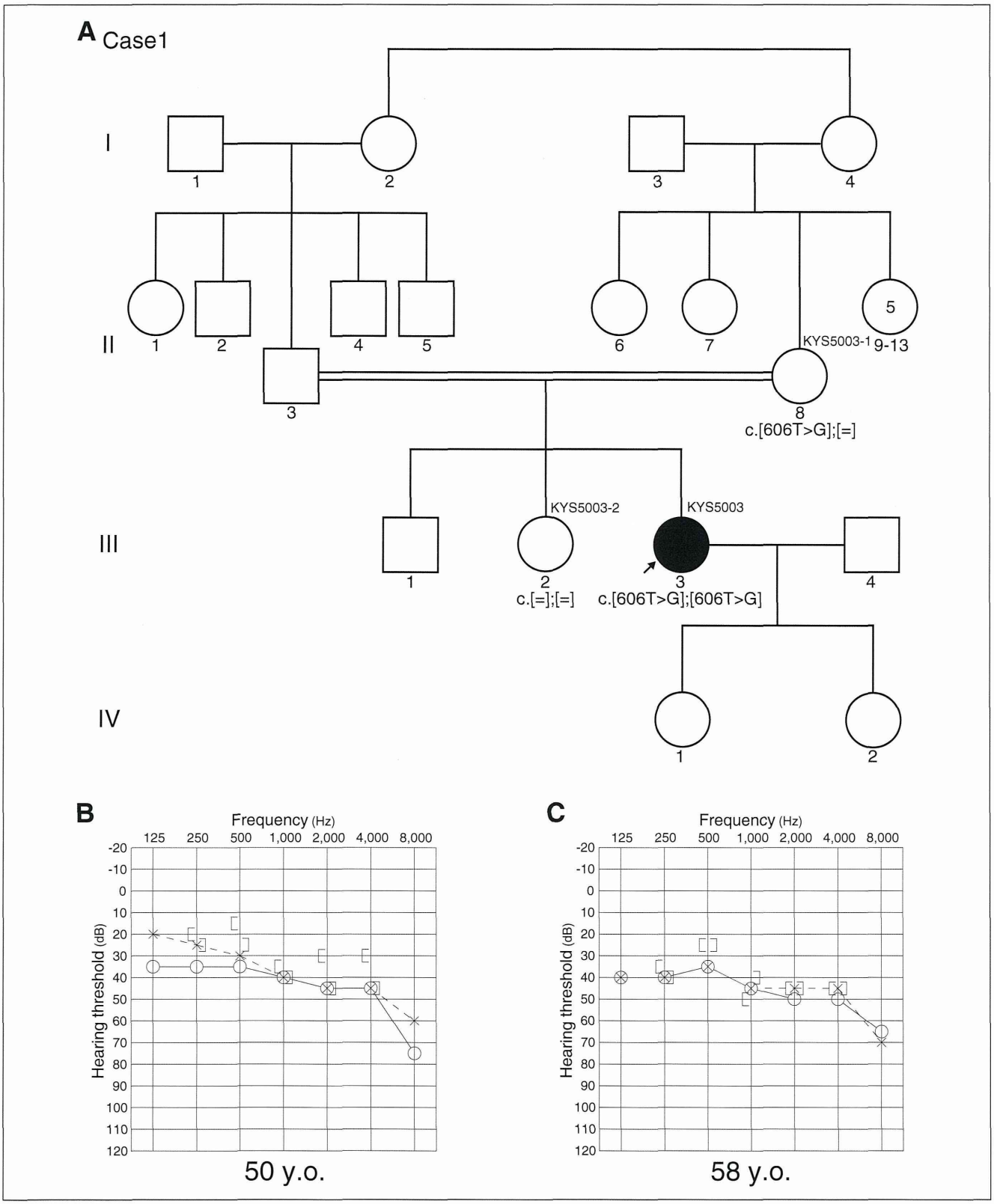

Figure 1. The pedigree and audiograms of the patient ID: KYS5003. (A) Pedigree and sequence results for the proband and family. (B) Audiogram of the proband revealed mild hearing loss at age 50. (C) Audiogram of the proband revealed similar mild hearing loss at age 58.

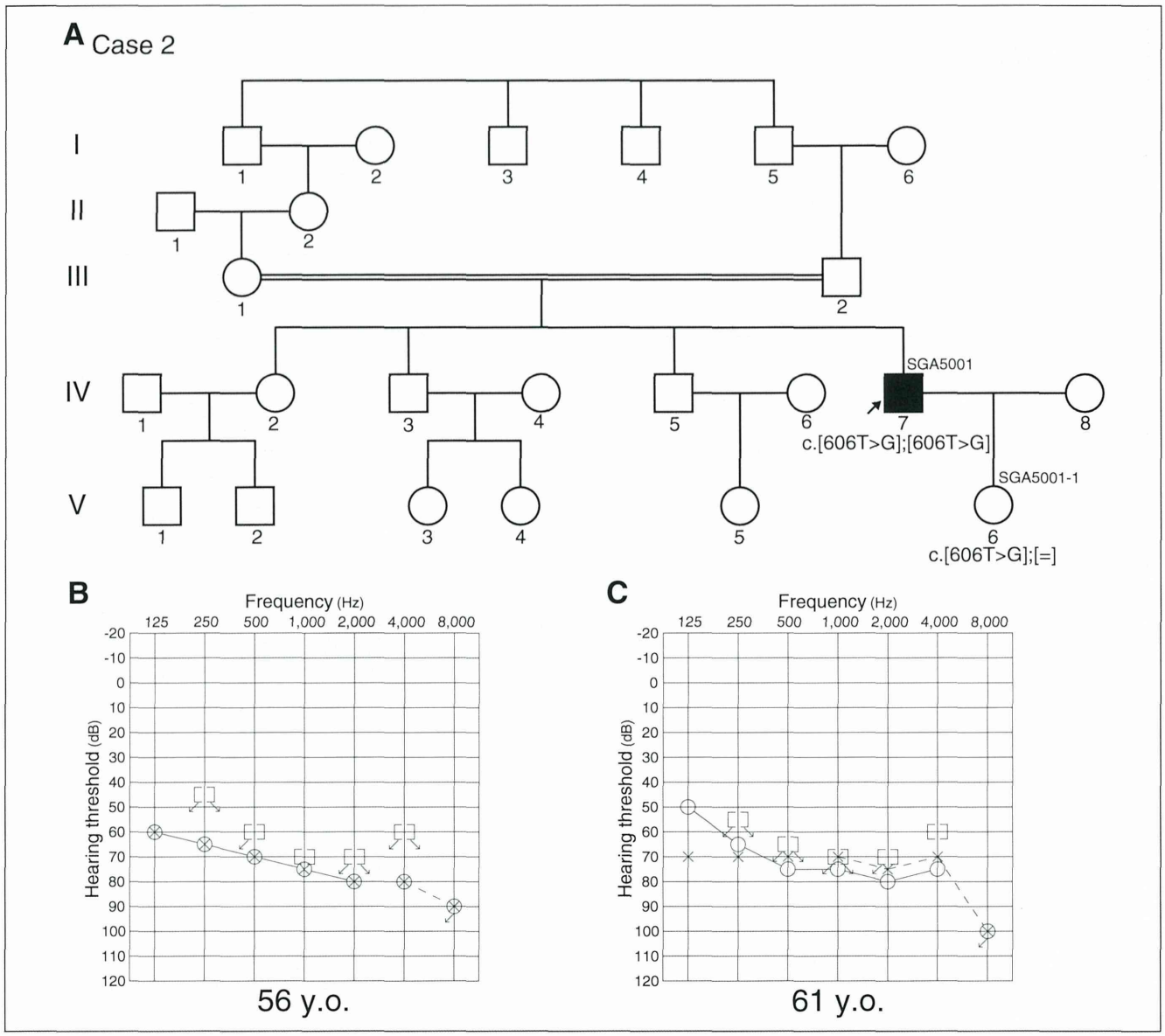

Figure 2. The pedigree and audiograms of the patient ID: SGA 5001. (A) Pedigree and sequence results for the proband and family. (B) Audiogram of the proband revealed severe hearing loss at age 56. (C) Audiogram of the proband revealed similar severe hearing loss at age 61.

employed functional prediction software (Polyphen2, SIFT, Mutation Taster, and LRT), which indicated the mutation to be damaging (0.99, 0.07, 0.99 and 1.00, respectively). The mutation was confirmed by segregation analysis in both families. For Case 1, her mother (II-8) was found to be a carrier for the mutation, but her older sister (III-2) had no mutation (Figure 1A). For Case 2, his child (V-6) was revealed to be a carrier for the mutation (Figure 2A).

Discussion

The present study identified a novel homozygous *CLRN1* mutation in both patients. The mutation (p.N202K) was strongly suspected to be pathogenic by several functional prediction software programs, and this mutation was not described in any mutation databases. Only 15 mutations in the *CLRN1* gene have been reported, and the missense mutation in this study is novel (Table 1). The *CLRN1* gene has at least 11 splice variants. The main variant encodes the clarin-1 protein consisting of 232 amino acid. Clarin-1 is a 4-transmembrane protein that is expressed in the hair cells of the organ of Corti and in the retina.[8,9] The function of clarin-1 remains unknown, however, the spatiotemporal expression pattern of clarin-1 in hair cells implicates its involvement in synaptic maturation.[10,11] Structural and sequence homology with the synaptic protein stargazin suggests a role of clarin-1