Mitochondrion 13 (2013) 791–794

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Phenotype and genotype of deaf patients with combined genomic and mitochondrial inheritance models

Shasha Huang [a,b,1], Guojian Wang [a,b,1], Yi Jiang [c,1], Yongyi Yuan [a,b], Dongyi Han [a], Yueshuai Song [a,b], Pu Dai [a,b,*]

[a] Department of Otolaryngology, PLA General Hospital, Beijing 100853, People's Republic of China
[b] Department of Otolaryngology, Hainan Branch of PLA General Hospital, Haitang Bay, Sanya 572000, People's Republic of China
[c] Fujian Medical University ShengLi Clinical College, Fujian Provincial Hospital, Fuzhou 350001, People's Republic of China

ARTICLE INFO

Article history:
Received 17 February 2013
Received in revised form 3 May 2013
Accepted 9 May 2013
Available online 18 May 2013

Keywords:
Sensorineural hearing loss
Autosomal recessive
Maternal inheritance
Different genes
Genetic model

ABSTRACT

In most studies, sensorineural hearing loss is reported as a single-gene disease with autosomal dominant or autosomal recessive or with X-linked or maternal inheritance. It is uncommon that the hearing impairment is caused by a combined inheritance model including genomic and mitochondrial models. Here, we report six patients with sensorineural hearing loss caused by co-existing mutations in *GJB2* or *SLC26A4* and the mitochondrial gene. And there was no significant difference in hearing phenotypes between the six patients and the controls. The results indicate the complicated genetic etiology of, and may impact the diagnostic strategy for, hereditary hearing impairment. All patient siblings will carry mitochondrial DNA A1555G or C1494T mutations, and 25% of siblings may carry the same homozygous or compound heterozygote mutations in *GJB2* or *SLC26A4*. Although this combined inheritance is not common in the Chinese deaf population (0.10%), our findings will have great impact in genetic counseling and risk prediction for deafness.

1. Introduction

Hearing impairment is the most common neurosensory disorder in humans. The reported incidence ranges from 1 in 300 to 1 in 1000 children (Downs, 1995; Mehl and Thomson, 1998, 2002). Approximately half of all cases have a genetic etiology, including syndromic and nonsyndromic forms, with extraordinary genetic heterogeneity. Nonsyndromic deafness accounts for 60–70% of inherited hearing impairment, and sensorineural hearing loss is reported as a single-gene disease in most studies. This involves more than 100 different genes with autosomal dominant (DFNA) or autosomal recessive (DFNB), or with X-linked (DFN) or maternal inheritance (Bitner-Glindzicz, 2002). In Mendelian inheritance, typical autosomal recessive sensorineural hearing loss is caused by mutations affecting both alleles of one gene, giving rise to a 25% recurrent risk in the siblings of the patient; and for maternal inheritance, is believed to a 100% recurrent risk in the siblings and matrilineal descendants. Mutations in the *GJB2* gene are the most common cause of nonsyndromic autosomal recessive sensorineural hearing loss (mild to profound) (Snoeckx et al., 2005). Two studies published in 1989 described a distinctive auditory phenotype associated with an enlarged vestibular aqueduct (EVA, which disease has been causally linked to mutations in the anion transporter gene *SLC26A4*, which encodes the protein pendrin) (Jackler and De La Cruz, 1989; Levenson et al., 1989). The hearing loss is predominantly sensorineural, variable in severity, fluctuating or progressive, symmetric or asymmetric, with a pre- or perilingual onset. Many EVA patients have evidence of a conductive hearing loss component associated with normal middle ear findings (Arjmand and Webber, 2004; Nakashima et al., 2000). The A1555G or C1494T mutations in *12S rRNA* have been implicated with aminoglycoside-induced and nonsyndromic hearing loss in families of different ethnic origins (Li et al., 2004a, 2004b; Tang et al., 2002). However, whether co-existing mutations in *GJB2* or *SLC26A4* and mitochondrial DNA have an impact on hearing loss has not been reported. This study examined six patients suffering from sensorineural hearing loss with bi-allelic mutations in *GJB2* or *SLC26A4* and co-existing A1555G/C1494T mutations in mitochondrial *DNA 12s rRNA* and analyzed the relationship among the hearing phenotypes and genotypes. The study will have great impact on genetic counseling and risk prediction.

2. Material and methods

2.1. Patients

Six hearing-impaired patients were selected from a cohort of 5934 patients with hearing impairment from unrelated Chinese families diagnosed in the Department of Otolaryngology, Chinese PLA General Hospital. A temporal CT scan, complete physical and otoscopic examinations, and an audiological study, including tympanometry, pure

* Corresponding author at: Department of Otolaryngology, PLA General Hospital, Do.28 Fuxing Road, Beijing 100853, People's Republic of China. Tel.: +86 1066937623; fax: +86 1068156974.
E-mail address: daipu301@vip.sina.com (P. Dai).
[1] These authors contributed equally to this article

1567-7249/$ – see front matter
http://dx.doi.org/10.1016/j.mito.2013.05.004

tone audiometry, or auditory steady-state response (ASSR), were performed in all patients. Cases 2 and 6 had clear histories of aminoglycoside antibiotic (Gentamicin) administration. Three patients were diagnosed with bilateral enlarged vestibular aqueduct syndrome but had an absence of goiter. Case 2 had a family history of hearing loss, but no family history of hearing loss was reported in the other five families (Fig. 1).

To compare differences in hearing, 272 patients were recruited from the Department of Otolaryngology, Chinese PLA General Hospital, including 100 patients with bi-allelic mutations in *GJB2*, 100 patients with EVA and identified bi-allelic mutations in *SLC26A4*, and 72 patients with either A1555G (63 individuals) or C1494T (nine patients) mutations in mitochondrial *12s rRNA*.

To determine whether it is common for pathogenic mutations to exist in genomic and mitochondrial genes simultaneously in the Chinese deaf population, an additional 5928 patients with sensorineural hearing loss (from unrelated families, with the exception of the six cases from the Genetic Testing Center of Otolaryngology Department of Chinese PLA General Hospital) were studied consecutively.

2.2. Clinical evaluation

The pure-tone average (PTA), calculated as the average of the thresholds measured at 0.5, 1.0, 2.0, and 4.0 kHz, was used to compare patient subgroups. The level of hearing loss in terms of PTA was described as follows: normal hearing, ≤20 dB; mild hearing impairment, 21–40 dB; moderate hearing impairment, 41–70 dB; severe hearing impairment, 71–90 dB; and profound hearing impairment, ≥91 dB.

2.3. Mutation analysis

After obtaining informed consent from the parents of each participant, genomic DNA was extracted from peripheral venous blood using standard procedures. Mutation screening was conducted using polymerase chain reaction (PCR) amplification, and exon 2 of *GJB2* was directly sequenced (Yuan et al., 2010). To examine whether other genetic defects were involved in the hearing loss in these subjects, the coding exons of *SLC26A4* (Wang et al., 2007) and mitochondrial *12S rRNA* (A1555G and C1494T mutations) (Zhao et al., 2004) were also sequenced.

2.4. Statistical analysis

The hearing level distribution in the different groups was evaluated using a nonparametric randomized block design. The Kruskal–Wallis rank test (K–W test) for independent samples was used to determine whether the observed hearing levels were significantly different among the various groups. All statistical methods were analyzed using SPSS (Statistical Package for the Social Sciences) 15.0 software. A *P*-value of 0.05 or less was accepted as statistically significant.

3. Results

As shown in Table 1, audiological evaluations showed that all the six patients in this study had moderate to profound symmetrical sensorineural hearing loss. Case 2 had a flat hearing curve with moderate hearing loss (PTA; 67 dB HL). The other five patients demonstrated severe to profound hearing loss (PTA; 98, 101, 93, 98, and 107 dB HL, respectively). *GJB2*, *SLC26A4*, and mitochondrial *12s rRNA* were sequenced in six patients (Table 1). Two patients (cases 1 and 2) were found to carry c.235delC homozygous mutations or c.235delC/c.427C > T (p.R143W) compound heterozygote mutations in *GJB2*, respectively. Three patients (cases 3, 4, and 5), who had enlarged vestibular aqueducts, were found to carry c.919-2A > G/c.2027 T > A (p.L676Q), c.919-2A > G/2168A > G(p.H723P), and c.919-2A > G/c.589G > A(p.G197R) compound heterozygote mutations in *SLC26A4*, respectively. All five patients also carried the A1555G homoplasmy mutation in mitochondrial *12s rRNA*. The sixth patient (case 6) was determined to carry c.235delC/c.512insAACG compound heterozygote mutations in *GJB2* and the C1494T homoplasmy mutation in mitochondrial *12s rRNA*.

For the 272 patients with different mutations in the three control groups (*GJB2*-related, *SLC26A4*-related, and *12S rRNA*-related hearing loss), the hearing levels and average PTAs of the different groups are shown in Table 2 (94 dB HL, 96 dB HL, and 83 dB HL in *GJB2*-, *SLC26A4*-, and *12S rRNA*-related groups, respectively). Audiological evaluations showed that the sensorineural hearing loss ranged from mild to profound in these three groups; the percentages of patients with severe to profound hearing impairment were as follows: 86% of patients with *GJB2* mutations, 87% of patients with *SLC26A4* mutations, and 73.6% of patients with the mitochondrial *12s rRNA* mutation.

Statistical analysis showed significant differences in the hearing level among the control groups ($P = 0.000$; $P < 0.05$). The hearing level in the *12S rRNA*-related group was significantly better than that in the other two control groups, and the hearing level in the *GJB2*-related hearing loss group was worst among these groups. No significant difference was found between the patient group and any control group ($P = 0.182$, $P = 0.499$, and $P = 0.202$; all $P > 0.05$).

In the other 5928 patients of this cohort, most genetic deafness was attributed to mutations in *GJB2*, *SLC26A4*, mitochondrial *12s rRNA*, or other genes, but no patient carried co-existing double-loci mutations in two or more genes through different genetic models, except for one patient with EVA, which was identified as a compound heterozygote for c.257C > G (p.T86R) and c.299-300delAT mutations in *GJB2* and c.1229C > T (p.T410M) and c.1079C > T (p.A360V) compound heterozygote in *SLC26A4*, both of which follow the autosomal recessive genetic model (Huang et al., 2013).

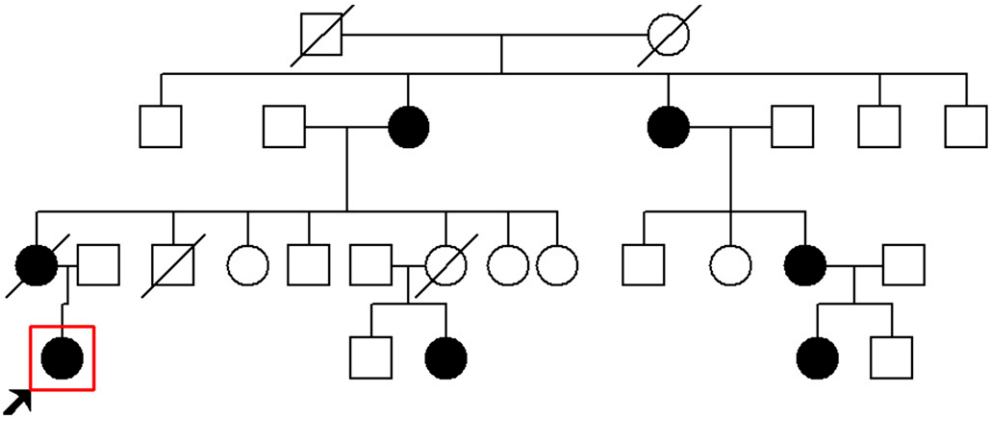

Fig. 1. Case 2 pedigree. Matrilineal relatives with deafness in this family. The relatives did not have a hearing test and did not undergo genetic testing.