Prevalence of mitochondrial gene mutations among hearing impaired patients

Shin-ichi Usami, Satoko Abe, Jiro Akita, Atsushi Namba, Hideichi Shinkawa, Masanori Ishii, Satoshi Iwasaki, Tomoyuki Hoshino, Juichi Ito, Katsumi Doi, Takeshi Kubo, Takashi Nakagawa, Sohtaro Komiyama, Tetsuya Tono, Shizuo Komune

Abstract

The frequency of three mitochondrial point mutations, 1555A→G, 3243A→G, and 7445A→G, known to be associated with hearing impairment, was examined using restriction fragment length polymorphism (RFLP) analysis in two Japanese groups: (1) 319 unrelated SNHL outpatients (including 21 with aminoglycoside antibiotic injection history), and (2) 140 cochlear implantation patients (including 22 with aminoglycoside induced hearing loss). Approximately 3% of the outpatients and 10% of the cochlear implantation patients had the 1555A→G mutation. The frequency was higher in the patients with a history of aminoglycoside injection (outpatient group 33%, cochlear implantation group 59%). One outpatient (0.314%) had the 3243A→G mutation, but no outpatients had the 7445A→G mutation and neither were found in the cochlear implantation group. The significance of the 1555A→G mutation, the most prevalent mitochondrial mutation found in this study of a hearing impaired population in Japan, among subjects with specific backgrounds, such as aminoglycoside induced hearing loss, is evident. (J Med Genet 2000;37:38-40)

Keywords: mitochondria; point mutation; hearing impairment; frequencies

Mitochondrial mutations have been shown to be responsible for syndromic as well as non-syndromic hearing impairment (see hereditary hearing loss home page, http://dnalab-www.uia.ac.be/dnalab/hhh/).

The 1555A→G point mutation is associated with a susceptibility to aminoglycoside antibiotics. In addition, in recent studies, several hearing impaired patients bearing the 1555A→G mutation who had no history of aminoglycoside injection were reported. It is likely that among the hearing impaired population there are a great number of subjects with the 1555A→G mutation.

The 3243A→G mutation has been reported in a high proportion of patients with clinical features of MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes). This mutation was also found in patients with diabetes mellitus and hearing loss is known to be associated with this mutation.

The 7445A→G mutation was reported to be associated with hearing loss in Scottish, New Zealand, and Japanese families. Palmo-planter keratoderma was also found in the New Zealand and Japanese families. On the basis of the growing genetic evidence stated above, attention should be paid to the genetic background of patients who visit clinics. However, to date, little is known about the frequency of this mutation among the hearing impaired population. In the present study, the frequency of three mitochondrial mutations among the hearing impaired population in Japan was evaluated.

Subjects and methods

The frequency of three mitochondrial point mutations, 1555A→G, 3243A→G, and 7445A→G, known to be associated with hearing impairment, was examined in two groups. All subjects gave informed consent to participation in the project. Group 1 consisted of 319 unrelated subjects with a mean age of 40.7 years (range 3-92) who visited outpatient clinics because of sensorineural hearing loss (SNHL). A history of aminoglycoside injection was present in 21 subjects and 21 were syndromic with diabetes mellitus. There were no other associated neurological signs. The mean age of onset was 32.0 (range 0-79). Twenty five subjects had a family history with one or more other family members having hearing loss (12 compatible with autosomal dominant, 11 with autosomal recessive, two with mitochondrial inheritance). The mean hearing level of these subjects was 58.6 dB; 37.7% of the subjects had mild hearing loss (up to 49 dB), 34.5% had moderate (50-69 dB), 9.6% severe (70-89 dB), and 18.2% profound (90 dB and over). Group 2 consisted of 140 subjects who had received cochlear implantation because of profound hearing loss, including 22 subjects with aminoglycoside induced hearing loss.

The frequency of three mitochondrial mutations was examined using restriction fragment length polymorphism analysis (RFLP). The PCR products were also sequenced by means of an ABI sequencer 377XL (Perkin Elmer Co Ltd).

Screening for the 1555A→G mitochondrial mutation has been previously described. In brief, mitochondrial DNA was extracted from the blood and nucleotide 1252-1726 amplified by PCR. Digestion was performed with a restriction enzyme (Alu26I) and the digested sample then electrophoresed on an agarose gel. As seen in fig 1A, normal controls had two
fragments, while in mutated DNA fragments loss of the Alu26I site, caused by the 1555 mutation, resulted in a single fragment. The 1555 mutation was confirmed by direct sequencing using an ABI sequencer 377XL (Perkin Elmer Co, Ltd).

To detect the 3243A→G mutation, nucleotide 3160-3333 was amplified by PCR. Digestion was performed with a restriction enzyme (Apal), and the digested sample then electrophoresed on an agarose gel. Mutated DNA fragments were digested into two fragments caused by the 3243 mutation (fig 1B). The 3243 mutation detected by RFLP analysis was confirmed by direct sequencing. All experiments included positive controls.

To detect the 7445A→G mutation, nucleotide 7178-7840 was amplified by PCR. Digestion was performed with a restriction enzyme (XbaI), and the digested sample then electrophoresed on an agarose gel. Normal controls had two fragments (fig 1C), whereas mutated DNA fragments should be found as a single band.

### Results

Table 1 summarises the results obtained in the present study.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SNHL</td>
<td>(SNHL+AG)</td>
</tr>
<tr>
<td>1555A→G</td>
<td>11/319</td>
<td>(7/21)</td>
</tr>
<tr>
<td>3243A→G</td>
<td>1/319</td>
<td>(1/21)</td>
</tr>
<tr>
<td>7445A→G</td>
<td>0/319</td>
<td></td>
</tr>
</tbody>
</table>

SNHL: patients who visited outpatient clinics for sensorineural hearing loss.

CI: patients who received cochlear implant.

+AG: patients with history of aminoglycoside injection.

DM: patients with diabetes mellitus.

The 1555A→G mutation was found in one patient in group 1, a frequency of 0.314% (1/319) in unrelated hearing impaired outpatients, and 4.76% (1/21) in the patients with diabetes mellitus. This patient had been referred to our clinic because of bilateral hearing loss associated with diabetes.

The 7445A→G mutation was not found in the present subjects.

### Discussion

The data presented suggest that the 1555A→G mutation is one of the most important mutations among the hearing impaired population in Japan, and approximately 3% of patients with SNHL possess this mutation. The significance of this mutation among the subjects with the specific background of aminoglycoside induced hearing loss is evident. Therefore, it should be noted that this mutation is the most probable candidate for the gene responsible for hearing impairment in patients with aminoglycoside exposure. Two subjects had a family history on the maternal side, suggesting that family history should also be considered, but other factors, such as age or severity of hearing loss, are not significant criteria for the selection of patients to undergo mtDNA analysis. Although the mean age of onset in patients with the 1555A→G mutation is younger than in those patients without the mutation, this is accounted for by the early age of aminoglycoside exposure.

This study showed a high frequency of this mutation among the deaf population with a history of aminoglycoside injection. It should be noted that profound hearing loss is possibly preventable and care must be taken when using aminoglycoside antibiotics. A rapid screening method as well as careful counselling should be widely instituted to prevent aminoglycoside induced hearing loss. Aminoglycoside antibiotics are still in use, especially in parts of Asia. The most common cause of hearing loss in China is aminoglycoside induced SNHL. The newly developed aminoglycoside antibiotics, which have less serious side effects, should also be used with caution as two subjects with the 1555A→G mitochondrial mutation were found to have experienced hearing loss after short term, therapeutic dosage exposure to them. Genetic backgrounds should be adequately checked before aminoglycoside antibiotics are used in patients from high risk populations.

The 1555A→G mutation was first reported in African and Asian populations, raising the possibility of a common ancestor for these groups. However, the results of recent phylogenetic analysis of 10 independent African and Asian families with the 1555A→G mutation and 13 families in Japan suggested that the 1555A→G mitochondrial mutation has multiple origins. In addition, this mutation has been recently found in Greek, English/ Irish, Italian,
Mexican, Puerto Rican, and Vietnamese, and Spanish and Cuban populations. The 1555A$\rightarrow$G mitochondrial mutation was also commonly found in Spanish hereditary hearing loss families (27.1% of all families and 55.9% of families with progressive deafness). This indicates that high risk populations may be found world wide.

It should be noted that a high frequency of the 1555A$\rightarrow$G mutation has been found in the profound hearing impaired populations. Of patients who received cochlear implantation because of aminoglycoside induced hearing loss, 57% had this mutation. We have reported good results with cochlear implantation in one patient with aminoglycoside induced hearing loss and the 1555A$\rightarrow$G mutation.

A series of reports has suggested that this mutation also causes hearing loss even without aminoglycoside injection, although the hearing impairment in such cases is usually milder. In the present study, four out of 11 subjects (group 1) and one out of 14 subjects (group 2) bearing the 1555A$\rightarrow$G mutation did not have any history of aminoglycoside injection. Therefore, this mutation is an important disease causing mutation for non-syndromic hearing loss unrelated to aminoglycoside antibiotics.

Concerning the frequency of the 3243 mutation, it has been found in approximately 1% of patients with diabetes mellitus. According to Kadowaki et al., 61% of the diabetes patients with the 3243A$\rightarrow$G mutation have hearing loss. The present study provided the frequency (0.314%) of the 3243A$\rightarrow$G mutation in the hearing impaired population. Furthermore, this mutation was found more frequently (4.76%) in the patients who had diabetes mellitus. This mutation should be considered as a cause of hearing loss in the patients who have diabetes.

The absence of the 7445 A$\rightarrow$G mutation suggested that this mutation is not a major cause of hearing loss in the Japanese population.

In conclusion, genetic testing showed the frequency of three mitochondrial mutations among the hearing impaired population in Japan. The high frequency of the 1555A$\rightarrow$G mutation in hearing impaired populations, especially in those with specific backgrounds, indicates that genetic backgrounds should be adequately checked in these patients.

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