Mitochondrial Gene Mutation Is a Significant Predisposing Factor in Aminoglycoside Ototoxicity

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Purpose: Aminoglycoside-induced deafness caused by mutations in the mitochondrial 12S ribosomal RNA gene has been described in a number of Asian patients. The purpose of the current study is to analyze ethnically diverse patients in the United States with hearing loss after aminoglycoside exposure for presence or absence of these mitochondrial DNA mutations, and establish the frequency and clinical presentation associated with them.

Patients and Methods: Clinical histories, medical records, and blood samples were obtained from 41 unrelated American individuals with hearing loss after aminoglycoside exposure. DNA was extracted from the blood of these individuals, amplified by the polymerase chain reaction, and analyzed for mitochondrial ribosomal RNA gene mutations by allele-specific oligonucleotide hybridization, restriction fragment length polymorphism analysis, and sequencing.

Results: The nucleotide 1555 A → G mutation was identified in 7 of 41 individuals (17%). None of the other known mutations was found. The ethnic origin of the individuals with predisposing mutations included Caucasians, Hispanics, and Asians. Four of the 7 patients with the 1555 A → G mutation had a family history of aminoglycoside-induced ototoxicity. Particularly unexpected was the late onset of hearing loss in 3 of these patients, years after the aminoglycoside exposure. The 12S ribosomal RNA gene was sequenced in these patients, and a second sequence change that could be responsible for the milder phenotype was detected in 1 of the 3 patients.

Conclusion: These findings imply that a significant proportion of patients with aminoglycoside-induced ototoxicity harbor mutations in the 12S rRNA gene, which can be detected by DNA screening. Also, the majority of these hearing losses could have been easily prevented by the simple taking of a clinical history. In these individuals, a genetic susceptibility to the ototoxic effects of aminoglycosides can be diagnosed, and deafness can be prevented in maternal relatives by avoidance of these antibiotics.

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Aminoglycoside ototoxicity is one of the most common causes of acquired deafness. Although vestibulo-cochlear damage is nearly universal when high drug levels are present for prolonged periods, at lower drug levels there appears to be a significant genetic component influencing susceptibility to aminoglycoside ototoxicity. Numerous families have been described in which several individuals became deaf after exposure to aminoglycosides, and dramatic differences in susceptibility to these drugs between macaque and patas monkeys also suggest a genetic component.

We analyzed three Chinese families in which several individuals developed deafness after the use of aminoglycosides. The pattern of maternal inheritance in these pedigrees, the known effect of aminoglycosides on ribosomal translation ability, and the presence of resistance mutations in a range of prokaryotic and eukaryotic organisms, implicated the mitochondrial ribosomes, and in particular the mitochondrial-encoded 12S rRNA gene, as the most likely locus of such predisposition to toxicity. In all three families, a mutation was
identified in the 12S mitochondrial rRNA gene that affected a site known to be important both in the binding of aminoglycosides and in resistance to the antibiotic.\(^5\) Also, a small proportion of "sporadic" patients, without a positive family history for aminoglycoside ototoxicity, have this particular mutation.\(^6\) These findings were confirmed in two Japanese families and additional Chinese sporadic cases.\(^7\)

Subsequently, we described three other mutations in the 12S rRNA gene: a heteroplasmic nucleotide deletion/insertion mutation around nucleotide 961 and two homoplasmic mutations, which also appear to predispose to aminoglycoside ototoxicity.\(^6\) It appears at this time that, at least in China and Japan where aminoglycosides are commonly used to treat minor infections, nearly every case of familial aminoglycoside-induced ototoxicity and a small proportion of the sporadic cases has the 1555 mutation.

We have now identified 41 individuals in the United States with hearing loss after aminoglycoside exposure and present the clinical characteristics and molecular analysis of their mitochondrial 12S ribosomal RNA genes. The results indicate that 17% of them have the 1555 A→G mutation, that the hearing loss can occur long after the aminoglycoside exposure, and that the simple taking of a family history can prevent a significant proportion of aminoglycoside-induced ototoxicity.

**PATIENTS AND METHODS**

**Patient Ascertainment**

Subjects were identified from two major sources, the House Ear Institute (HEI) in Los Angeles, CA, and Gallaudet University (GU) in Washington, DC. All HEI medical records coded as "toxic" hearing loss were reviewed. This comprised a total of 300 charts spanning an 11-year period beginning in late 1984. Of these, 114 had documented or highly suggestive medical history of aminoglycoside exposure. Each patient in this group was sent a letter describing the project and soliciting participation. A single telephone call was attempted to all nonresponders with a U.S. address. Seven patients declined participation, 67 were unavailable (deceased, address or phone incorrect, no response to letter/phone message), and 38 agreed to be studied.

Two additional patients were found to be ineligible after review of more detailed history. Participants were solicited from the GU population via campus announcements and posters, and by placing notices in several publications for the hearing impaired. One additional patient was ascertained when he developed profound, unilateral sensorineural hearing loss (SNHL) immediately after undergoing intratympanic instillation of gentamicin for treatment of Meniere's disease in that ear. A final patient was ascertained via his wife's family history when she reported to our center for prenatal care. All samples were obtained with informed consent.

**Patient Characteristics**

The subjects comprised 18 males and 23 females with sensorineural hearing loss ranging in age from 7 to 81 years. There were 33 Caucasians (22 Europeans, 4 Eastern Europeans, 2 Mediterraneans, 1 middle Eastern, 4 unspecified), 5 Asians (2 Chinese, 1 Korean, 1 Laotian, 1 Vietnamese), 1 of mixed descent with a Chinese/Japanese father and a Caucasian mother, and 2 Hispanics (1 Mexican, 1 Puerto Rican). All patients reported bilateral hearing loss, except two cases with unilateral involvement including the patient with Meniere's disease. Onset of tinnitus or hearing loss was immediate or soon after the exposure in most cases, but occurred as late as 15 to 20 years after the exposure in several patients. The hearing loss was described as stable in 19 patients, progressive in 16, cyclic in one individual, and was not characterized in 5. The aminoglycoside was identified as streptomycin in 27 cases, gentamicin in 7, kanamycin in 3, gentamicin and amikacin in 1, and abdominal neomycin (by irrigation) in 1. In two cases, the specific drug could not be verified. Four patients had a clear history of aminoglycoside ototoxicity in other family members. Eight other subjects reported some family history of hearing loss including otosclerosis, hearing loss attributed to noise exposure or quinine exposure, presbyacusis, and unspecified hearing loss. None of these family members were suspected to have had an aminoglycoside exposure, but this cannot be eliminated with certainty. Ten subjects with a history of prematurity and/or low birth weight were exposed to aminoglycosides in infancy. Eight subjects reported concurrent illnesses that may have predisposed them to the ototoxic event, including 4 patients with renal disease/failure, 1 bone marrow transplant patient, 1 whose exposure occurred during treatment for a urinary tract infection, 1 with a high fever and rash, and 1 premature infant with Rh incompatibility. Thus, the entrance criteria were loosely defined as a likely history of aminoglycoside exposure followed by hearing loss and no definite other cause of hearing loss preceding the aminoglycoside exposure.

**DNA Preparation**

DNA was isolated from blood samples and from Epstein-Barr virus–transformed lymphoblastoid cell lines for some patients, by the method of Old.\(^9\)
Polymerase Chain Reactions

Mitochondrial DNA was amplified using 150 ng DNA, 10 pmol of each primer, 10 mmol/L Tris-hydrochloric acid, pH 8.3, 50 mmol/L potassium chloride, 1.5 mmol/L magnesium chloride, 200 μmol/L deoxynucleotides triphosphates, and 1U Taq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT) in a volume of 25 μL, with an initial 5 minute denaturation at 95°C, followed by 40 cycles of 94°C, 30 seconds; 54°C, 1 minute; 65°C, 3.5 minutes in an MJ Research (Watertown, MA) PTC-100 programmable thermal controller.

Single nucleotide reactions were compared in adjacent lanes to screen for the diagnostic stuttering pattern seen previously. For patients 025, 034, and 039, the entire 12S rRNA gene was amplified and sequenced, as described.

RESULTS

Allele-specific oligonucleotide hybridizations were performed as described.

Restriction Fragment Length Polymorphism (RFLP) Analysis

One hundred fifty nanograms of PCR-amplified DNA (between nucleotides 1092 and 3377) was digested with 10 U BsmAI (New England Biolabs, Inc, Beverly, MA). After electrophoresis through 1.5% agarose, the gel was stained with ethidium bromide and visualized on an ultraviolet illuminator.

DNA Sequence Analysis

Sequence analysis used the CircumVent thermal cycle DNA sequencing kit (New England Biolabs, Inc) and α35S-dATP (NEN/Dupont, Wilmington, DE). For the 961ΔT mutation, patient mtDNAs were amplified between nucleotides 816-1899, and sequenced with the primer 816-835. Single nucleotide reactions were compared in adjacent lanes to screen for the diagnostic stuttering pattern seen previously. For patients 025, 034, and 039, the entire 12S rRNA gene was amplified and sequenced, as described.

RESULTS

We found the homoplasmic 1555 A → G mutation in the 12S ribosomal RNA gene in seven cases, using allele-specific oligonucleotide hybridization and RFLP analysis (Fig 1). In 40 of the 41 cases, DNA from blood was initially analyzed, and then DNA obtained from the lymphoblastoid cell lines was used to confirm the results. In patient 040, we had found the mutation independently in her brother (not included in the 41 patients), and thus did not do the confirmatory test. No discrepancy between the analysis of DNA from blood and lymphoblastoid cell lines was found. None of the 41 individuals had the 1243, 1520, or the Δ961 mutations (screened by ASO hybridization and sequence analysis, data not shown). The clinical characteristics of these cases are summarized in Table 1. The ethnic background of individuals with mutations in the mitochondrial 12S ribosomal RNA gene included Caucasians of several different countries of origin, Hispanics, and Asians. Six of the seven cases were female, and all of them had received streptomycin. None of these individuals received aminoglycosides in early infancy (no known exposure before the age of 2 years). The histories were fairly dramatic in

Fig 1. Identification of 1555 mutation by restriction enzyme analysis. MtDNA from aminoglycoside-induced deaf patients was amplified with primers flanking the mutation, between nt 1092 to 3377 and digested with BsmAI. The 717 base pair fragment is an internal control for enzyme activity. Normal DNA results in 462 base pair and 1106 base pair fragments, whereas loss of the BsmAI site caused by the 1555 A → G mutation results in a 1568 base pair fragment. Lanes 1 to 11 correspond to patients 024 to 034.
### TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Patient Age (y)</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Drug Diagnosis</th>
<th>Hearing Threshold (Right/Left) (dB)</th>
<th>Family History of Aminoglycoside Ototoxicity</th>
<th>Patient Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>004</td>
<td>40 F</td>
<td>Greek</td>
<td>S</td>
<td>Immediately after treatment for streptococcal adenopathy. Diagnosed at 7 y.</td>
<td>85/95, 95/105, 95/105</td>
<td>Sister with SNHL immediately after streptomycin at 3 y.</td>
<td>GU</td>
</tr>
<tr>
<td>011</td>
<td>44 F</td>
<td>English/Irish</td>
<td>S</td>
<td>Suspected after treatment for adenopathy. Diagnosed at 7 y.</td>
<td>30/25, 85/90, 100/100</td>
<td>Multiple family members became deaf after various streptomycin exposures.</td>
<td>GU</td>
</tr>
<tr>
<td>014</td>
<td>43 F</td>
<td>Italian</td>
<td>S</td>
<td>Immediately after treatment for fever at 3 y.</td>
<td>85/90, 105/110</td>
<td>Two maternal first cousins deaf after streptomycin.</td>
<td>GU</td>
</tr>
<tr>
<td>025</td>
<td>81 F</td>
<td>Mexican</td>
<td>S</td>
<td>Slowly progressive SNHL after treatment for suspected tuberculosis at 47 y.</td>
<td>75/70, 105/105, 120/110</td>
<td>No relatives with known aminoglycoside exposures.</td>
<td>HFI</td>
</tr>
<tr>
<td>034</td>
<td>66 M</td>
<td>Puerto Rican</td>
<td>S</td>
<td>SNHL diagnosed 17 y after treatment following foot surgery.</td>
<td>10/10, 65/65, 90/85</td>
<td>None. Patient and multiple relatives have Charcot-Marie-Tooth disease.</td>
<td>HEI</td>
</tr>
<tr>
<td>039</td>
<td>57 F</td>
<td>Chinese/Japanese/Caucasian</td>
<td>S</td>
<td>Immediate tinnitus after treatment for pneumonia at 17 y.</td>
<td>15/20, 10/15, 55/55</td>
<td>None. No relatives with known aminoglycoside exposures.</td>
<td>HEI</td>
</tr>
<tr>
<td>040</td>
<td>20 F</td>
<td>Vietnamese</td>
<td>S</td>
<td>Immediately after treatment for malaria at 4 y.</td>
<td>80/85, 80/90, 90/90</td>
<td>Brother with SNHL after streptomycin at 4 y. Mother's SNHL attributed to streptomycin exposure.</td>
<td>HEI</td>
</tr>
</tbody>
</table>

Abbreviations: NR, no response; S, streptomycin.

...five cases in whom tinnitus and/or hearing loss occurred very soon after aminoglycoside exposure. In the other two cases, and in 1 of the 5 patients with the initial symptom of tinnitus, slight hearing loss was noted early after exposure and progressed very slowly, with a significant level of impairment only after more than a decade following the exposure. Four of the seven cases had a positive family history in maternal relatives, most of whom had clearly-described aminoglycoside exposure. Many of these family members also described severe hearing loss shortly following the exposure. Of note, there was some variability among affected family members, for example patient 011, who reports profound SNHL in one affected individual in her kindred, whereas she and other affected relatives have some preservation of low-tone hearing. All of the subjects with a family history of ototoxic deafness were found to have the 1555 mutation, and none of the subjects with other causes of familial hearing loss had the 1555 mutation. One of the patients (025) had a congenitally deaf paternal first cousin, who was not exposed to aminoglycosides during fetal life or infancy. Her history of progressive later-onset SNHL clearly followed immediately after an aminoglycoside exposure. This patient's history is further compounded in that she also describes low-grade noise exposure. Patient 034 also has an unusual medical history in that he, his father, older sister, daughter, and three other family members are affected with Charcot-Marie-Tooth disease. However, none of the other affected individuals have any known hearing loss and none have any known aminoglycoside exposures, thus implicating the aminoglycoside as the cause of the hearing loss in case 034.
Severity of the hearing loss was generally severe to profound through the speech range and at higher frequencies, though hearing was relatively better preserved in case 039. Three of the cases with the 1555 mutation (025, 034, 039) had initially very mild, and then slowly progressive hearing loss for many years after the aminoglycoside exposure. The mtDNA of patient 025 failed to hybridize to either oligonucleotide probe used to screen for the 1520 mutation. We sequenced her mtDNA in this region to look for a possible compensatory mutation. We identified a homoplasmic C→T sequence change at position 1525, which is the terminal nucleotide of the penultimate stem loop of the 12S rRNA gene and is unpaired (Fig 2). The impact of this change on aminoglycoside sensitivity is not known. To test whether individuals 025, 034, and 039 had other nucleotide changes in the 12S rRNA gene that might influence the impact of the 1555 mutation, the gene was sequenced in all three individuals. The known polymorphic site at nt 1438 was G in two of the individuals and A in the third. Otherwise, no other sequence change was found within the 12S rRNA gene of individuals 034 and 039.

The findings among the subjects without the 1555 mutation were on average different from those seen in the cases described above. All instances of prematurity/low birth weight were in this group (including the patient with Rh incompatibility). Therefore, if the 1555 cases are excluded, 10 of 34 (29%) of these subjects received aminoglycosides in infancy and had other significant potential etiologies for hearing loss. Similarly, the 5 patients with renal disease, 1 bone marrow transplant patient (who may have received multiple nephrotoxic drugs), a child treated for a urinary tract infection at 6 weeks of age, and another child with high fever and a rash were also in this group. Nevertheless, most of the remaining subjects described typical, dramatic onset of tinnitus followed or accompanied by SNHL occurring shortly after aminoglycoside exposure in a generally healthy individual. Of note are three subjects, all otherwise healthy young men, who reported immediate deafness after aminoglycoside treatment for severe food poisoning, burns received in a tanker truck fire, and a traumatic amputation of a finger, respectively. Although they may have had extenuating circumstances, including dehydration in the first two cases (neither reports any renal compromise following the ototoxic event), these are as convincing histories of ototoxicity as any in the group of patients with the 1555 mutation. None of these patients has any other known family history of ototoxic hearing loss.

**DISCUSSION**

We screened the mitochondrial DNA of 41 U.S. patients with hearing loss after aminoglycoside exposure for the known mutations in the 12S ribosomal RNA gene that have been associated with predisposition to aminoglycoside ototoxicity. 5,8 Despite the fact that the selection criteria were very loosely defined, a significant proportion of patients with the 1555 mutation were identified. The number of 7 of 41 needs to be contrasted with the fact that
the 1555 mutation was not found in over 400 controls\textsuperscript{2} nor in a number of molecular screening tests of hearing impaired populations. None of the other three suggested predisposing mutations in Asian patients\textsuperscript{8} was identified in this sample. This report thus extends the original finding of aminoglycoside susceptibility mutations to non-Asian populations, demonstrates the surprising potential of initially very mild but then slowly progressive hearing loss after aminoglycoside exposure in individuals with the 1555 mutation, supports the notion of additional genetic predisposing genes to aminoglycoside ototoxicity, and points to the importance of taking a family history before aminoglycoside administration.

Our original descriptions of aminoglycoside-induced ototoxicity in Asian patients with the 1555 mutation seemed to indicate that the hearing loss occurred after small outpatient doses of the drug and with relatively immediate onset of severe hearing loss. The clinical criteria for selecting patients in these studies were quite stringent. In the current study, selection criteria were loosely defined. Thus, for example, the three individuals with significant hearing loss only many years after the original aminoglycoside exposure, but no other diagnosed cause for the hearing deficit, were initially included with little hope for detecting a susceptibility mutation. However, the finding of the 1555 mutation in these three individuals indicates that the presence of the mutation can initiate a long-term process that culminates eventually in hearing loss. The mechanism of this can be identical to the one postulated for the immediate onset hearing loss, namely mitochondrial protein mistranslation,\textsuperscript{9} with accumulation of abnormal proteins over many years because of a persistent presence of the aminoglycoside, or alternatively an additional long-term mechanism may potentiate the original insult. Either way, the genotype-phenotype relationship between the 1555 mutation and aminoglycoside-induced ototoxicity is more complex than initially thought.

Several of the patients had quite dramatic histories of hearing loss immediately after aminoglycoside exposure as young adults with an acute injury history but did not have any of the susceptibility mutations found in the 12S rRNA gene. This supports the possibility that other gene mutations can also predispose to aminoglycoside ototoxicity. Aminoglycoside toxicity mechanisms unrelated to mitochondrial ribosomes have been proposed by other investigators,\textsuperscript{12} and genes in these pathways could certainly have predisposing mutations. If these mutations act in an autosomal recessive fashion, then the family history in these cases would be expected to be negative.

The most remarkable finding from the point of view of clinical relevance is the fact that 4 of the 7 individuals with the 1555 mutation had a positive family history for aminoglycoside-induced ototoxicity. It is the clinician’s responsibility to inquire about such a history before prescribing these antibiotics.

ACKNOWLEDGMENT

We thank the physicians and patients who have contributed to this study.

REFERENCES