Mutation in the mitochondrial 12S rRNA gene in two families from Mongolia with matrilineal aminoglycoside ototoxicity

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Abstract
Irreversible hearing loss is a catastrophic complication of treatment with aminoglycoside antibiotics such as streptomycin, gentamycin, and kanamycin. Many kindreds showing a matrilineal pattern of inheritance of this trait have been described in China where the widespread use of aminoglycoside antibiotics accounts for approximately 25% of profound deafness in some districts. Because of the characteristic inheritance pattern, mitochondrial DNA (mtDNA) mutations were postulated to be the cause of the deafness in these pedigrees. In 1993 it was shown that an A to G substitution at base pair 1555 of the mitochondrial 12S ribosomal RNA gene was the only mutation common to all the families with aminoglycoside ototoxicity.

We ascertained three Mongolian pedigrees from the School for the Deaf and Blind in Ulaanbaatar, all of which contained multiple affected subjects with streptomycin induced deafness in a pattern consistent with matrilineal transmission. Amplified mtDNA, obtained from transformed lymphoblastoid cell lines using previously described primers, showed the A to G point mutation in the 12S rRNA gene in two of the three families by restriction analysis as well as direct sequencing. No other example of this substitution was found among 400 control samples from Mongolians with normal hearing.

We have thus confirmed the clinical relevance of the 1555 A to G mitochondrial mutation in the 12S rRNA gene by identifying it in affected subjects with familial aminoglycoside ototoxicity in another ethnic group. In countries where aminoglycosides are widely used, genetic counselling and screening of high risk families before the use of these drugs could have a dramatic effect on the incidence of deafness.

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Hereditary deafness is a heterogeneous group of genetic entities which can be distinguished by their clinical manifestations, mode of inheritance, and map location. In 10–20% of cases, deafness is associated with clinical features which permit the diagnosis of a specific syndrome while the remaining cases are considered non-syndromic. Most non-syndromic deafness (60-70%) shows a recessive pattern of inheritance, 20-30% of cases show dominant transmission, and 2-3% are X linked.

Matrilineal or mitochondrial transmission of aminoglycoside induced deafness was first suggested by Higashité after studying two Japanese families and a review of several Chinese pedigrees reported earlier. Jaber et al. studied a large Israeli-Arab kindred with hereditary non-syndromic deafness where the deafness could be traced back five generations to one common female ancestor. Interestingly, based on segregation analysis, they proposed a digenic model with one locus being mitochondrial, and the other autosomal recessive. A similar pattern of matrilineal transmission has been seen in several smaller Chinese pedigrees of aminoglycoside induced deafness. In both the digenic non-syndromic deafness pedigree and the families with aminoglycoside induced deafness, a homoplasmic mitochondrial ribosomal RNA (rRNA) A to G substitution at nucleotide position 1555 was identified. This mutation occurs at a highly conserved region of the 12S rRNA gene, where aminoglycosides are known to bind and where mutations conferring aminoglycoside resistance have been described in other species. A small proportion of people with aminoglycoside ototoxicity but no family history of the disease also harbour the same rRNA mutation. Of the various other sequence changes reported by Prezant et al., the 663 A to G substitution in the 12S rRNA gene and the 1736 A to G substitution in the 16S rRNA gene were also present in the subjects they studied with the aminoglycoside induced toxicity. These variants were found in about 11% and 10% of the normal Asian population, respectively. Since four of their controls both with and without these mutations received streptomycin with no ill effects, these changes are unlikely to cause deafness by themselves.

In the present study, five subjects from three Mongolian families (fig 1) were screened for the 663 A to G and 1555 A to G changes in the 12S rRNA gene, and for the 1736 A to G change in the 16S rRNA gene. The families were ascertained through probands at the School for Deaf and Blind in Ulaanbaatar,
Mongolian subjects with normal hearing were sent as 5 mm blood spots on a filter paper. For isolation of DNA from blood spots we used the organic extraction method described by Desalle et al with slight modification. Our yield from the latter was in the range of 7 to 15 \( \mu g/\text{ml} \). The mutation at 1555 A to G has been described as the most invariant in people with streptomycin induced deafness. To study this change in our samples, DNA was amplified in a Perkin Elmer 9600 thermal cycler with primers described by Prezant et al. The reaction mixture included 500 ng DNA, 10 pmol of each primer, 200 umol each dNTP mix, 1.5 mmol MgCl\(_2\), and 2.5 U Taq polymerase in a final volume of 25 \( \mu l \). PCR conditions for amplification were as follows: initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation (95°C, one minute), annealing (60°C, one minute), and extension (72°C, one minute 30 seconds). Ten micrometers of the amplified product were subjected to restriction enzyme digestion with Alw26I, since the 1555 A to G mutation abolishes the restriction site for this enzyme. Undigested samples show a 1605 bp product (labelled a in fig 2B). In contrast, digested samples normal controls show three bands, b, c, d, which are approximately 206, 293, and 1106 bp in length while affected subjects exhibit only two bands, 206 and 1399 bp in size (bands b and c, respectively). Products were analysed by electrophoresis in 1.5% Seakem LE agarose (FMC bioproduct). As seen in fig 2A, four of the five affected subjects have only two bands approximately 206 and 1399 bp in size compared to the three bands observed in digested samples from normal controls. The nature of the substitution at the Alw26I site was subsequently identified by direct sequencing of the PCR product using the Sequenase PCR product sequencing kit marketed by USB (fig 3A). The substitutions at nt663 and nt1736 were also tested for by direct sequencing of PCR amplified products (fig 3B, C). Interestingly, all three subjects from the first family and subject 12 from the third pedigree had both of these mutations, while subject 10 from the second pedigree had neither. Thus, although subject 12 in family 3 did not carry the 1555 A to G mutation, she had both of the variant changes. To date, all those with aminoglycoside ototoxicity studied for molecular changes have shown the 1555 A to G mutation. Thus, the absence of this well-defined mutation in subject 12 with matrilineal transmission of the aminoglycoside ototoxicity is unique and highly suggestive that other mitochondrial mutations or nuclear genes could be involved in this disorder. In keeping with this possibility Prezant et al recently reported the isolation of a novel yeast gene that confers neomycin resistance to yeast transformants. The father in this pedigree has deafness secondary to trauma; however, the mother and the two children all have a history of aminoglycoside induced deafness.

In order to evaluate the apparent association between these mutations and the aminoglycoside induced hearing loss and to determine if they are polymorphic in the Mongolian popu-
Results were normal in a number of subjects between 1262 and 1625 of the amplified product. The A to G substitution is denoted by the arrow. (B) Identification of the 1736 A to G change in the 16S rRNA gene by direct sequencing. Results on subject 3, affected with sensorineural deafness, from family 1 and a normal control by direct sequencing between nucleotides 1226 and 1900 of the amplified product. The A to G substitution is denoted by the arrow. Results were confirmed on the reverse strand and in two different experiments. (C) Identification of the 663 A to G substitution by allele specific oligonucleotide hybridisation. DNA from affected subjects (Nos 3, 6, 7, 10, and 12) and normal controls hybridised to the normal and mutated oligonucleotides. All subjects except No 10 hybridised with the mutated oligonucleotide indicating that they harbour the mutation.

Table 1 Summary of mutations in the three pedigrees and normal controls

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<tr>
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<th>1555 A to G</th>
<th>663 A to G</th>
<th>1736 A to G</th>
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<tbody>
<tr>
<td>Family 1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Family 2</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Family 3</td>
<td>0/400</td>
<td>17/400</td>
<td>18/400</td>
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We analysed samples from 400 control subjects from Mongolia who had normal hearing by anamnestic history. DNA isolated from 400 blood spots on filter paper was amplified and subjected to restriction endonuclease digestion for the 1555 A to G change. The 663 A to G and 1736 A to G substitutions were detected by the hybridisation of amplified mitochondrial DNA with normal and mutant oligomers using a modification of the method for allele specific oligonucleotides described by Thein et al. The oligonucleotides used to detect the 663 change were 20 base pairs from nucleotide 654 to 673; for the 1736 substitution it was 19 base pairs from 1727 to 1745. The 1555 A to G change was absent in all these samples (fig 2B), while the 663 and 1736 nt substitutions were detected in 17/400 and 18/400 of the samples respectively (table 1). Fourteen subjects with the 663 change also carried the 1736 change.

We concluded that the 1555 A to G substitution, which is located in the highly conserved region of the 12S rRNA mitochondrial gene, is responsible for aminoglycoside sensitivity in two of the three families. Within the limits of our testing the mutations were homoplasmic in both families. This mutation was absent in the 400 controls from Mongolia. The two other mutations, 663 A to G and 1736 A to G, which are found with a frequency of 10-11% in the Chinese population, were detected in four of the five affected subjects we studied, but only 4-5% of the Mongolian sample with normal hearing. The mutations were detected together in two of the three affected families, but only 3.5% of the control subjects who had normal hearing by history ($\chi^2=16.8, 1$ df, p$\leq 0.001$). The population of Mongolia numbers about 2 million, of whom a quarter live in Ulanbatar and another 15% in much smaller urban areas. At least 40% of the rural population still lead nomadic lives as they have for centuries. The population is divided into at least 15 ethnic subgroups, the largest of which (the Hoth) accounts for about 70% of the total population. Although the distribution and frequency of the 1555 A to G change within these subgroups remains to be determined, the avoidance of the use of aminoglycosides by the matrilineal relatives of subjects with drug induced ototoxicity would provide at least one effective strategy for reducing the incidence of deafness in the Mongolian population.

