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

A Pandya, X Xia, J Radnaabazar, J Batsuuri, B Dangaansuren, N Fischel-Ghodsian, W E Nance

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 Ulanbaatar, 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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Keywords: mitochondrial 12S rRNA gene; Mongolia; matrilineal aminoglycoside ototoxicity.

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 Higashi 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 with both of 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 Ulanbaatar,
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 μg/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/lnNTP mix, 1.5 mmol/l MgCl₂, and 2.5 U Taq polymerase in a final volume of 25 μl. 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 micrograms 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 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 e, 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 Genentra Systems Inc. Additional samples from 400

Mongolia. Blood samples from 14 subjects from three independent families were received, and immortalised lymphoblastoid cell lines were successfully established on five of them. DNA was isolated from cells using the Puregene DNA isolation kit marketed by Gentra Systems Inc. Additional samples from 400

Figure 1 Pedigrees of the three families.

Figure 2 Restriction endonuclease digestion of DNA from (A) affected probands and (B) normal subjects. (A) shows subjects 3, 6, 7, 10, and 12 under lanes 2 to 6, a normal control in lane 1, and a 1 kb ladder as a molecular marker in lane M. All except subject 12 harbour the 1555 A to G mutation. (B) shows DNA from blood spots of normal unaffected people from Mongolia, undigested in lane 1 and 3 and digested in lanes 2 and 4 to 10. None of the samples studied shows this mutation.
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Family

Summary of

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and normal controls

Table 1

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<th>1555A to G</th>
<th>663A to G</th>
<th>1736A to G</th>
</tr>
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<tbody>
<tr>
<td>Family 1</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Family 2</td>
<td>+</td>
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</tr>
<tr>
<td>Family 3</td>
<td>0/400</td>
<td>17/400</td>
<td>18/400</td>
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<tr>
<td>Controls</td>
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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 (χ²=16.8, 1 df, p<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.


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