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New polymorphic mtDNA restriction site in the 12S rRNA gene detected in Tunisian patients with non-syndromic hearing loss

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ABSTRACT

The 12S rRNA gene was shown to be a hot spot for aminoglycoside-induced and non-syndromic hearing loss since several deafness-associated mtDNA mutations were identified in this gene. Among them, we distinguished the A1555G, the C1494T and the T1095C mutations and C-insertion or deletion at position 961. One hundred Tunisian patients with non-syndromic hearing loss and 100 hearing individuals were analysed in this study. A PCR-RFLP analysis with HaeIII restriction enzyme showed the presence of the A1555G mutation in the 12S rRNA gene in only one out of the 100 patients. In addition, PCR-RFLP and radioactive PCR revealed the presence of a new HaeIII polymorphic restriction site in the same gene of 12S rRNA site in 4 patients with non-syndromic hearing loss. UVIDOC-008-XD analyses showed the presence of this new polymorphic restriction site with a variable heteroplasmic rates at position +1517 of the human mitochondrial genome. On the other hand, direct sequencing of the entire mitochondrial 12S rRNA gene in the 100 patients and in 100 hearing individuals revealed the presence of the A750G and A1438G polymorphisms and the absence of the C1494T, T1095C and 961insC mutations in all the tested individuals. Sequencing of the whole mitochondrial genome in the 4 patients showing the new HaeIII polymorphic restriction site revealed only the presence of the A8860G transition in the MT-ATP6 gene and the A4769G polymorphism in the ND2 gene.

Several mitochondrial DNA (mtDNA) mutations were found to be associated with sensorineural hearing loss [1,2]. Most of the molecular defects responsible for this disorder are mutations in the 12S rRNA gene and tRNA genes. In particular, the 12S rRNA gene was shown to be a hot spot for aminoglycoside-induced and non-syndromic hearing loss since several deafness-associated mtDNA mutations were identified in this gene. Among them, the A1555G mutation in the same highly conserved decoding site of the 12S rRNA was associated with both aminoglycoside-induced and non-syndromic hearing loss in many families of different ethnic backgrounds [3–13]. Similarly, the C1494T mutation in the same highly conserved decoding site of this rRNA was associated with both aminoglycoside-induced and non-syndromic hearing loss in a large Chinese family [14]. In addition, a C-insertion or C-deletion at position 961 of the 12S rRNA gene was shown to be associated only with aminoglycoside-induced deafness [15,16], while the T961G mutation was implied to be responsible for the non-syndromic hearing loss in five Caucasian pediatric subjects [9]. Furthermore, the T1095C mutation was also shown to be associated with hearing impairment [17,18].

In this report, we performed a mutational screening of the mitochondrial mutations in the 12S rRNA gene in 100 Tunisian patients with non-syndromic hearing loss. The results showed the presence of a new HaeIII polymorphic restriction site in 4 patients with variable heteroplasmic rates at position +1517 of the human mitochondrial genome. No mitochondrial mutation was observed in these 4 patients after the sequencing of the whole mitochondrial genome except the A860 and the A4769G polymorphisms. The results showed also the absence of the C1494T, T1095C and 961insC mutations in the 100 tested patients and the presence of the A1555G mutation in one patient.

Patients and methods

Patients

A total of 100 patients with non-syndromic hearing loss were analysed in this study. These patients belong to 100 unrelated Tunisian families having at least two affected subjects. No other abnormalities such as muscular or neurological defects were encountered indicating the presence of non-syndromic deafness in the studied families and no patient had a previous history of exposure to aminoglycosides. In addition, 100 Tunisian hearing individuals were tested as controls.

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Methods

DNA extraction. After getting informed consent from all the participating family members, the total DNA was extracted from peripheral blood using phenol chloroform standard procedures [19].

Screening of the mitochondrial A1555G mutation. Screening for the A1555G mutation was carried out using PCR-RFLP. PCR amplification of a 339 bp DNA fragment of the 12S rRNA gene containing the mutation was performed using primers as previously reported [7]. The PCR reaction was carried out using 200 ng DNA, 6 pmol of each primer, 2 mM $MgCl_2$, 500 μM dNTP, 1× PCR buffer and 2 U Taq DNA polymerase in a final volume of 50 μL. The PCR reaction was performed in a GeneAmp PCR System 9700 (Applied Biosystems) thermal cycler with an initial denaturation during 5 min at 95 °C, followed by 35 cycles of 94 °C for 30 s; 54 °C for 1 min and 65 °C for 2 min and ending with a final extension at 72 °C for 10 min.

After PCR amplification, 35 μL of PCR product were digested with 10 U of the HaeIII restriction endonuclease (Amersham pharmacia biotech), separated through 2% agarose gel and visualised with ethidium bromide in UV. In the wild allele, digestion resulted in two fragments of 218 bp and 121 bp. The A1555 G mutation created a novel HaeIII restriction site and the digestion resulted in three fragments of 218, 91 and 30 bp.

A radioactive PCR was used to quantify the percentage of mutant mtDNA by adding 1 μL of [α-P32] dATP to the PCR reaction mixture just before the last PCR cycle. After HaeIII digestion, the digestion product was electrophoresed through a 12% non-denaturing polyacrylamide–bisacrylamide gel.

Digestion resulting fragments were analysed with a UVIDOC-008-XD analyser to quantify the rate of heteroplasmy.

Screening of the mitochondrial C1494T, T1095C and 961insC mutations. Searching for the C1494T, T1095C and 961insC mutations in the 12S rRNA mitochondrial gene was performed by direct sequencing. The entire mitochondrial 12S rRNA gene was PCR amplified in two overlapping fragments using two couples of primers corresponding to the mitochondrial genome at positions 545–564 and 1251–1232 for the first couple and 1133–1152 and 1752–1705 for the second one.

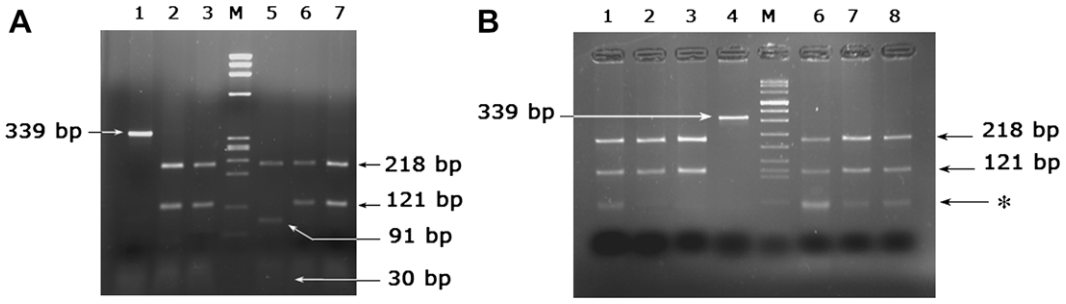

Fig. 1. Results of PCR-RFLP analysis of Tunisian patients with hearing loss: A 339 bp PCR fragment is digested with HaeIII. The wild-type mtDNA is cleaved into two fragments of 218 bp and 121 bp in length, whereas PCR product containing the A1555 G mutation is cleaved into three fragments, 218 bp, 91 bp and 30 bp in length. (A) Lane 1: Undigested PCR product, lane 2 and 3: digestion in patients non-harbouring the A1555G mutation, M: DNA Ladder phi 174 HaeIII digest; lane 5: digestion in patient carrying the A1555G mutation, lane 6 and 7: digestion in control individuals. (B) Lane 1, 6, 7 and 8: digestion in patients with hearing loss showing new polymorphic mtDNA restriction HaeIII site, lane 2: digestion in patient with deafness without the polymorphic restriction HaeIII site, lane 3: digestion in a healthy control; M: DNA Ladder phi 174 HaeIII digest.

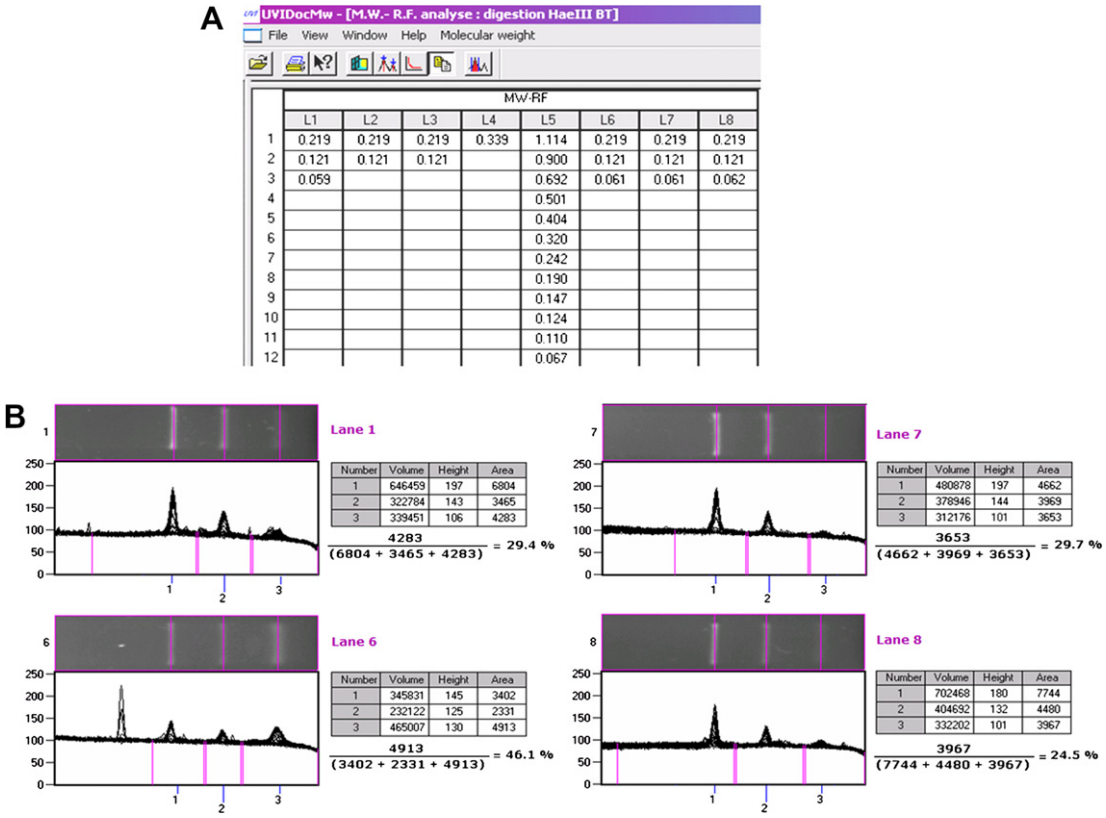

Fig. 2. (A) Determination of the size of the fragment generated by the new polymorphic restriction HaeIII site with the UVIDOC-008-XD analyser in the four Tunisian patients with non-syndromic hearing loss. (B) Quantification of the heteroplasmic levels of the new polymorphic restriction HaeIII site with the UVIDOC-008-XD analyser in the four Tunisian patients with non-syndromic hearing loss.