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Prevalence of the mitochondrial A 1555G mutation in Moroccan patients with non-syndromic hearing loss

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ABSTRACT

Mutations in mitochondrial DNA (mtDNA), especially the A1555G transition in the 12S rRNA gene, are one of the causes of both aminoglycoside-induced and non-syndromic sensorineural hearing loss.
Objective: The aim of this study was to determine the prevalence of the A1555G mitochondrial mutation in Moroccan patients.
Methods: We performed molecular characterization by PCR-RFLP and direct sequencing of one hundred and sixty four patients (84 unrelated familial and 80 sporadic cases) with a congenital sensorineural non-syndromic hearing loss and one hundred normal hearing controls for the occurrence of the A1555G mutation.
Results: Mutational analysis of the mtDNA showed the presence of the homoplasmic A1555G mutation in three families, leading to a frequency of 3.6% similar to that reported for European-populations. No A1555G mutation was detected in sporadic and controls cases. However, we detected in twenty normal hearing controls a novel polymorphism A1557C, which was not found in patient samples. We further evidenced the presence of the A1438G mitochondrial polymorphism in four patients with sensorineural hearing loss and in five controls.
Conclusion: Our results show that the occurrence of the A1555G mutation in hearing impaired patient's accounts for 3.6% in a Moroccan patients and those novel mtDNA polymorphisms might contribute to a novel sub-haplogroup specific of the Magrheb.

1. Introduction

Sensorineural hearing loss is a very common congenital disorder affecting 1 in 1000 newborns [1,2]. In the paediatric population, 50% of patients with deafness have a genetic cause, with autosomal dominant, autosomal recessive, X-linked, or mitochondrial patterns of inheritance [3,4]. Hearing loss can result from a mutation in a single gene or from a combination of mutations in different genes. Hearing loss can also be caused by environmental factors, including perinatal infection, acoustic or cerebral trauma affecting the cochlea, or ototoxic drugs, such as aminoglycoside antibiotics, or it can be a result of interactions between genetic and environmental factors [3,4]. Specifically, in familial cases of ototoxic sensorineural hearing loss, the aminoglycoside hypersensitivity is often maternally transmitted, suggesting the involvement of mutations in mitochondrial DNA (mtDNA) [5], which have been found associated with both aminoglycoside-induced and non-syndromic deafness [5,6]. Among the identified non-syndromic deafness-causing mtDNA mutations, the A7445G [7,8], 7472insC [9,10] and T7510C changes have been reported in the tRNASer [11–13]. In addition, the A1555G mutation has been found to be responsible for non-syndromic deafness in many families of different ethnic backgrounds [11,13–17]. In the absence of exposure to aminoglycosides, the A1555G mutation produces a clinical phenotype that varies considerably among family members, ranging from moderate progressive, severe congenital deafness to hearing loss with a late onset [13,15], to completely normal hearing [13–15]. Mitochondrial mutations associated to non-syndromic deafness are often homoplasmic or at high levels of heteroplasmy, suggesting that high threshold of the mutated mtDNA must accumulate for pathogenicity. Variable phenotypic expression of these mtDNA mutations requires the contribution of other factors such as nuclear modifier genes, environmental factors, or other mitochondrial mutations.

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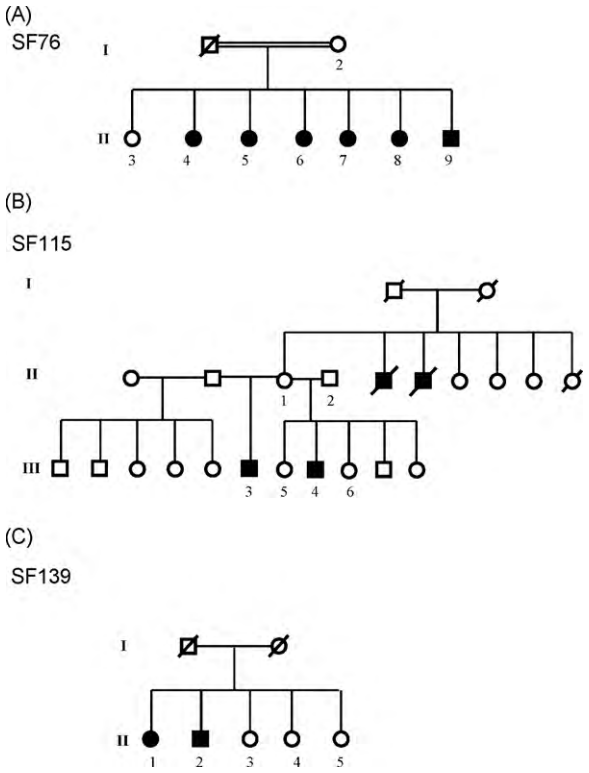

Fig. 1. Pedigree of the three Moroccan families segregating for the A1555G mutation in the mitochondrial 12S rRNA gene all individuals with hearing loss shows A1555G mutation and the mothers are normal hearing carriers. A: SF76 family, B: SF115 family and C: SF139 family.

Several studies were performed in Moroccan patients with sensorineural hearing loss to describe genetic abnormalities in nuclear genes such as *GJB2*, *GJB3*, *GJB6*, *ESPN* and *MYO7A* [18–20], but to date no study was undertaken to evaluate mitochondrial DNA mutations impact in the 12S rRNA gene in hearing loss. Here, we report the first mutational screening of the A1555G mitochondrial mutations in Moroccan patients with sensorineural hearing loss to assess the prevalence of this mutation.

2. Patients and methods

2.1. Patients

We ascertained and analysed 84 unrelated families (in which 198 individuals were affected) and 80 sporadic cases from different regions of Morocco with non-syndromic sensorineural hearing loss. All families had at least two deaf subjects. No other abnormalities were encountered indicating the non-syndromic feature of the disease. In addition, one hundred Moroccan normal hearing individuals were tested as controls. All patients were previously tested negatives in GJB2, GJB3 and GJB6 genes [20]. Participating members of families and sporadic cases underwent general otological examinations and pure-tone audiometry with air and bone conduction at 250, 500, 1000, 2000, 4000, and 8000 Hz.

The patients participating in this study, or their parents when minors, were explained extensively the reason of the genetic survey and they all delivered a written informed consent.

2.2. Methods

Total DNA was extracted from the patient's peripheral blood using the classical phenol/chloroform protocol.

Screening for the A1555G mutation was carried out using PCR-RFLP as previously described [14]. PCR amplification of a 339 bp DNA fragment of the *12S rRNA* gene containing the mutation was performed using the forward primer 5′-GCTCAGCCTATATACCGC-CATCTTCAGCAA-3′ and the reverse primer 5′-TTTCCAGTACACT-TACCATGTTACGACTTG-3′.

The PCR amplification was performed in a thermal cycler (GeneAmp PCR System 2700 Applied Biosystems) in a final volume of 25 μl using 200 ng of DNA, 8 pmol of each primer, 2 mM $MgCl_2$, 500 μM dNTP, 10× PCR buffer, and 2 units of Taq DNA polymerase. The condition for PCR amplification was as follows: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation (94 °C, 1 min), annealing (56 °C, 1 min), extension (72 °C, 1 min 20 s), and a final extension at 72 °C for 5 min.

The PCR products were digested with 10 units of the restriction endonuclease HaeIII (Promega), separated through 2% agarose gel and visualized with ethidium bromide in UV light. Digestion of the wild allele resulted in two fragments of 218 and 121 bp. The A1555G mutation created a novel HaeIII restriction site and the digestion resulted in three fragments of 218, 91 and 30 bp, respectively.

2.3. Sequencing

The results of PCR-RFLP were confirmed by direct sequencing. After purification by incubation with exonuclease I and shrimp alkaline phosphatase, the PCR product of all patients were directly sequenced on both strands using the ABI-Prism Big Dye terminator cycle sequencing Ready Reaction kit V3.1 (ABI-Prism/Applied Biosystems) and analyzed on an ABI-Prism 3130 Genetic Analyser (Applied Biosystems). The sequences were compared to the wild-type coding sequence of *12S rRNA* gene using the ABI SecScape software version 2.5.

3. Results

We detected the A1555G mutation by PCR-RFLP in three out of the 84 tested families (Figs. 1 and 2). This result was confirmed by direct sequencing of the corresponding PCR product (Fig. 3). The analysis of all members of these families confirmed the presence of

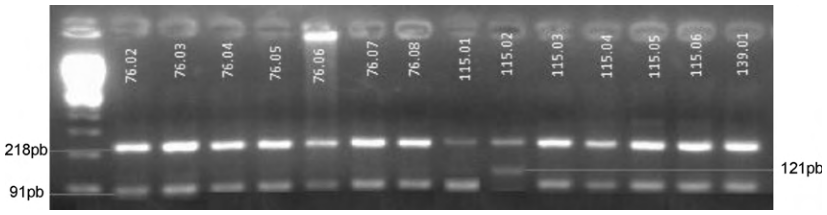

Fig. 2. Results of PCR-RFLP analysis of Moroccan patients with hearing loss: a 339 bp PCR fragment is digested with HaeIII. The wild-type mtDNA is cleaved into two fragments, 218 bp, and 121 bp in length. Whereas PCR product containing the A1555 G mutation is cleaved in to three fragments, 218, 91 and 30 bp in length. The first family SF76: digestion in patients harbouring the A1555G mutation, The second family SF115: digestion in patient carrying the A1555G mutation, M: DNA Ladder phi 174 HaeIII digest.