J Hum Genet (1999) 44:304–307

ORIGINAL ARTICLE

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Rapid mass screening method and counseling for the 1555A→G mitochondrial mutation

Received: March 25, 1999 / Accepted: April 16, 1999

Abstract The 1555A→G point mutation is associated with a susceptibility to aminoglycoside antibiotics, and is of particular interest, as it may cause hearing loss even without aminoglycoside exposure. There may be a considerably large high-risk population in Japan, and to avoid possible side effects in this group, a rapid mass screening system and careful counseling are recommended. We are currently using the mutant allele specific amplification (MASA) method to detect the 1555A→G mitochondrial mutation and we distribute a warning card to subjects found to bear this mutation.

Key words Mitochondria · Hearing loss · 1555A→G mutation · Aminoglycoside antibiotics · MASA method

Introduction

Various mitochondrial mutations have been shown to be responsible for both syndromic and nonsyndromic hearing impairments (see hereditary hearing loss home page <http://dnalab-www.uia.ac.be/dnalab/hhh/>). The 1555A→G point mutation is known to be associated with a susceptibility to aminoglycoside antibiotics, and is of particular interest, as it may be related to inner ear susceptibility (Prezant et al. 1993). We have recently elucidated the clinical features of hearing loss due to this mutation (Usami et al. 1997). Our screening for the 1555A→G mutation among the hearing-impaired population has revealed a total of 128 subjects bearing it from 33 families, as of February, 1999. Although probands and a few family members had received aminoglycoside antibiotic injections and suffered subsequent hearing loss, most of the family members did not have significant hearing loss. However, those with the 1555A→G mitochondrial mutation are a high-risk population who may readily incur hearing loss after even a small dosage of aminoglycoside antibiotics. To avoid such possible side effects in the high-risk population, careful counseling is recommended. We are currently employing the mutant allele specific amplification (MASA) method to detect the 1555A→G mitochondrial mutation, and we distribute a warning card to subjects bearing this mutation.

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Subjects and methods

Subjects

DNA samples from 908 subjects, including patients with sensorineural hearing loss who visited outpatient clinics and their family members, were used in the present study. All subjects had given their informed consent to participate in the project.

Methods

Mitochondrial DNA was extracted from peripheral blood using a Genomix DNA isolation kit (Talent, Trieste, Italy). Both the MASA method and the PCR-restriction fragment length polymorphism (RFLP) method were performed on all samples. The PCR products which were shown to have the 1555A→G mutation by these two methods were sequenced to confirm the base change.

Mutant allele specific amplification (MASA) method

The reaction scheme is illustrated in Fig. 1A. The principle of MASA is based on exploiting the great difference in the

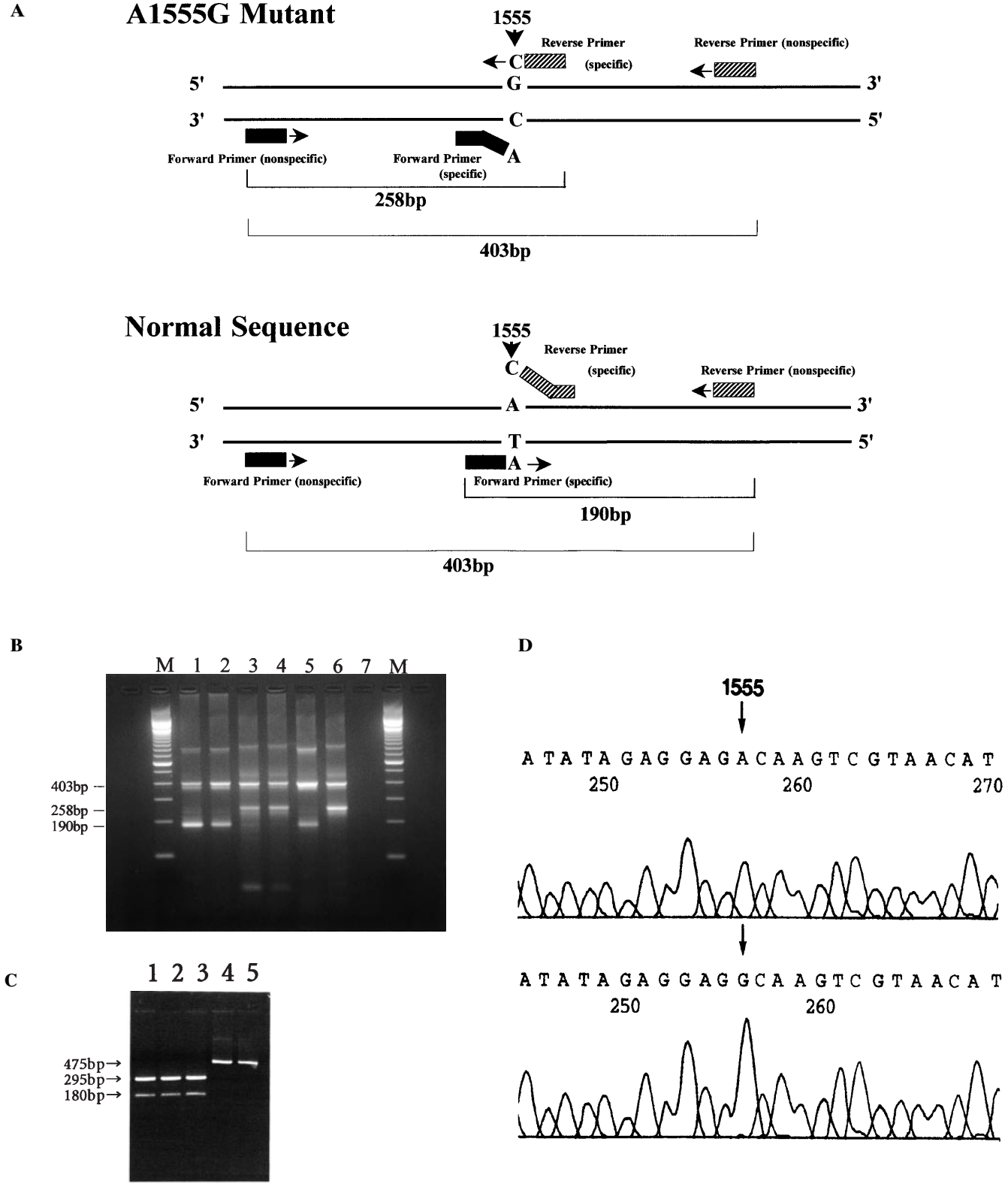

Fig. 1. A Strategy for mutant allele specific amplification (MASA) with competitive priming of mitochondrial DNA template bearing the 1555A→G mutation. Mutant type gives both 258-bp and 403-bp bands, while normal type gives both 190-bp and 403-bp bands. **B** MASA method. Ethidium bromide staining of PCR products for detection of the 1555A→G mutation. PCR products (10 μl) were electrophoresed in a 3% agarose gel containing 0.5 μg/ml ethidium bromide. *Lane M*, Molecular size marker (100-bp-ladder); *lanes 1*, *2*, 1555A→G mutation-negative clinical specimens; *lanes 3*, *4*, 1555A→G mutation-positive clinical specimens; *lane 5*, 1555A→G mutation-negative plasmid; *lane 6*, 1555A→G mutation-positive plasmid; *lane 7*, blank (no DNA control). **C** PCR-restriction fragment length polymorphism (RFLP) method. 1555A→G mutation presence (*lanes 4*, *5*). The restriction enzyme divides normal PCR products into two fragments (*lanes 1–3*). **D** Direct sequencing. Conversion of the nucleotide of 1555 from A to G is shown by sequencing results

efficiency of extension from the 3′ end of the primer, on primer-template complexes, either perfectly matched or mismatched at the 3′ end of the primer. In brief, the mutant mtDNA has a C/A mismatch at the 3′ end of the forward specific primer, and this mismatch prevents the annealing and the amplification of the sense strand. On the opposite DNA strand, the 3′ end of the reverse specific primer is complementary to the base in the target 1555 sequence. The PCR amplification of the mutant mtDNA will generate predominantly a 258-bp type-specific product that originates from the couple forward non-specific and reverse specific primers. Conversely, the normal mtDNA has an identical mismatch at the 3′ end of the reverse specific primer, and this mismatch prevents the polymerization of the antisense strand. A 190-bp product will be given, originating from the couple forward specific and reverse non-specific primers corresponding to the normal 1555 sequence. Irrespective of the 1555 mutation, a 403-bp product will originate from the non-specific couple of primers flanking the nucleotide of 1555 in addition to both type-specific products. Our MASA test is carried out simultaneously in a single reaction tube (50 µl of reaction mixture) containing both type-specific primer pairs (each 20 pmol), 500 ng of template DNA, 2.5 nmol each of dNTPs, 1.25 U of AmpliTaq Gold (Perkin-Elmer, Norwalk, CT, USA), buffered with 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl2, 0.1 mg/ml gelatin, at pH 8.3. The PCR is run with an initial heating step at 94°C for 15 min followed by 40 cycles, each consisting of 94°C for 30 s, 63°C for 30 s, and a final extension at 72°C for 7 min, in a Model PJ-2000 Thermal Cycler (Perkin-Elmer). After PCR, 10 µl of the amplification product is mixed with 2 µl of a gel loading buffer (containing sucrose and bromophenol blue) and electrophoresed on 3% Agarose X (Nippon Gene, Tokyo, Japan). Gels are stained with ethidium bromide, and photographed using a UV transilluminator (Fig.1B).

RFLP analysis and direct sequencing

Screening for the 1555A→G mitochondrial mutation has been confirmed by two previously described methods (Usami et al. 1997). In brief, the nucleotide of 1252 through 1726 is amplified by PCR. Digestion is performed with a restriction enzyme (*Alw*26I), and the digested sample is then electrophoresed on an agarose gel. As seen in Fig. 1C, normal controls have two fragments, while in mutated DNA fragments, loss of the *Alw*26I site, caused by the 1555 mutation, results in a single fragment. The PCR products are also sequenced by means of an ABI sequencer 377XL (Perkin Elmer). Figure 1D shows the nucleotide of a patient in which 1555 was converted from A to G.

診察を受ける前にこの券を医師に見せてください

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Please show this card to the doctor before undergoing any examinations.

Drug Use Warning Card

Name＿＿＿＿＿＿＿＿＿＿＿＿ Birthdate＿＿＿＿＿＿＿＿＿＿＿＿

This patient has a high-risk of suffering hearing loss from **aminoglycoside antibiotic** use.

Department of Otorhinolaryngology, Hirosaki University Hospital
Phone: Outpatient Clinic 0172-39-5277/Department Office 39-5099

Fig. 2. Warning card (*upper panel*, Japanese card; *Lower panel*, English translation)

Results

In the present study, 68 subjects were diagnosed as having the 1555A→G mutation by the MASA method and the PCR-RFLP method (Table 1). All of the 68 samples were directly sequenced and the mutations were confirmed. One sample showed a discrepancy by comparison with the PCR-RFLP method (MASA-positive, PCR-RFLP-negative). Direct sequencing did not show the 1555A→G mutation, but showed that the discrepancy was due to two polymorphisms (1709G→A, 1716T→C) in the primer site.

Warning cards and counseling

Following confirmation of a 1555A→G mutation, a warning card is distributed to the patient. These cards include the patient's name and birth date and a warning, as well as the address and phone numbers of our hospital (Fig. 2). Patients/families bearing the 1555A→G mutation are also counseled about the risk of aminoglycoside antibiotics.

Discussion

Because of their broad spectrum of antibacterial activity and their cost-effectiveness, aminoglycoside antibiotics are still widely used, especially in a number of Asian countries. The leading type of hearing loss in China is aminoglycoside-induced sensorineural hearing loss (SNHL) (Fu 1985; Lu

Table 1. Comparison between the MASA method and the PCR-RFLP method

		MASA	
		+	−
PCR-RFLP	+	68	0
	−	1	839

Although no false-negatives were seen with the MASA method, one false-positive occurred due to the polymorphisms in the primer site
MASA, mutant allele specific amplification; PCR-RFLP, polymerase chain reaction — restriction fragment length polymorphism

1987). It is true that, because of the considerable side effects and the development of viable alternatives, aminoglycoside usage has decreased in many countries, including Japan. However, a new generation of aminoglycoside antibiotics with minor side effects, which has been introduced in Japan and Europe, should also be used with due caution. We recently experienced two subjects bearing the 1555A→G mitochondrial mutation who had hearing loss after short-term, therapeutic dosage exposure to these new aminoglycoside antibiotics (Usami et al. 1998); Therefore, genetic backgrounds should be carefully checked before the use of aminoglycoside antibiotics for individuals in high-risk populations. We are currently investigating the degree of risk in various populations. Preliminary data suggest that approximately 3% of patients with SNHL possess the 1555A→G mutation (S. Usami, unpublished data).

This mutation was first reported within populations in restricted areas, including Arab-Israeli, Chinese, and Japanese (Prezant et al. 1993; Hutchin et al. 1993). However, recent reports demonstrate that this mutation is also found in Greek, English/Irish, Italian, Mexican, Puerto Rican, and Vietnamese (Fischel–Ghodsian et al. 1997); Zairian (Matthijs et al. 1996); Spanish (Estivill et al. 1998); and Mongolian populations (Pandya et al. 1997). Furthermore, phylogenetic analysis of ten independent families with the 1555A→G mutation from Africa and Asia (Hutchin and Cortopassi 1997) and 13 families in Japan (Abe et al. 1998) suggested that the 1555A→G mutation occurred sporadically and multiplied through the evolution of the mtDNA, indicating that the 1555A→G mutation may exist all over the world.

The mutant allele specific amplification (MASA) method is a relatively simple one, in which only one PCR reaction is needed per sample and additional processes, such as enzyme restriction, are not necessary. Therefore it is suitable for mass screening (Takeda et al. 1993; Hayashi et al. 1994). In the present study, the MASA method's acceptable reliability compared with the other methods was confirmed (Table 1). No false-negatives were found in our screening series. One sample showed a false-positive, due to a polymorphism in the primer site. Misdiagnosis can be avoided by doing additional RFLP analysis for subjects with positive results.

Genetic background should be considered to avoid aminoglycoside-induced hearing loss. Molecular genetic testing will help to evaluate the high-risk population and enable genetic counseling of subjects bearing the 1555A→G mutation.

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