J Hum Genet (2003) 48:480–483
DOI 10.1007/s10038-003-0056-9

SHORT COMMUNICATION

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Prevalence of the mitochondrial DNA A1555G mutation in sensorineural deafness patients in island Southeast Asia

Received: 12 May 2003 / Accepted: 30 June 2003 / Published online: 3 September 2003

Abstract A mtDNA A1555G base substitution in a highly conserved region of the 12S rRNA gene has been reported to be the main cause of aminoglycoside induced deafness. This mutation is found in approximately 3% of Japanese and 0.5–2.4% of European sensorineural deafness patients. We report a high prevalence (5.3%) of the A1555G mutation in sensorineural deafness patients in Sulawesi (Indonesia). Our result confirms the importance of determining the prevalence of the mtDNA A1555G mutation in different populations, and the need for mutation detection before the administration of aminoglycoside antibiotics.

Keywords Sensorineural deafness · Aminoglycoside antibiotics · Mitochondrial DNA

Introduction

Amongst mutations that underlie sensorineural deafness, an A1555G base substitution in the small (12S) *rRNA* gene of mtDNA is of particular interest as a main cause of antibiotic-induced deafness (Fischel-Ghodsian, 1999). This mutation increases sensitivity to aminoglycoside ototoxicity, but has been reported also in deaf individuals who have not been exposed to these antibiotics (Fischel-Ghodsian, 1999). It has been detected in sensorineural deafness patients with widely differing ethnic backgrounds (Pandya et al. 1997; Casano et al. 1998; Estivill et al. 1998; Scrimshaw et al. 1999; Kupka et al. 2002; Ostergaard et al. 2002; Tekin et al. 2003), with a prevalence of 0.5–2.4% in European sensorineural deafness patients (Scrimshaw et al. 1999; Kupka et al. 2002; Ostergaard et al. 2002; Tekin et al. 2003).

In Japan, approximately 3% of sensorineural deafness patients and 10% of cochlear implantation patients carry the mtDNA A1555G mutation (Usami et al. 2000), leading to the suggestion that testing for the mutation should be carried out as a routine before the administration of aminoglycoside antibiotics. The A1555G mutation has been reported also in China (Prezant et al. 1993), and recently in a large Balinese family with non-syndromic sensorineural deafness (Malik et al. 2003), raising the possibility that the mutation might also be an important cause of sensorineural deafness in other populations of Asia. We have thus investigated the prevalence of this mutation amongst sensorineural deafness patients in the populations of the island Southeast Asia, focusing in the first instance on that of South Sulawesi in the middle of the large Celebes archipelago.

Patients and Methods

Two groups of sensorineural deafness patients who came to the ENT clinic in the Wahidin Hospital in Makassar, South Sulawesi, were investigated: 75 unrelated non-syndromic sensorineural deafness patients (age 6–43 years; group I), and 50 unrelated patients with diabetes mellitus (DM) and sensorineural deafness (age 40–60 years; group II). In addition, we examined 100 normal healthy unrelated individuals as normal control subjects. All group II patients were confirmed to have Type 2 diabetes following the WHO criteria, and receiving treatment for diabetes mellitus at the hospital. Informed consent to participate in the study was obtained, and the study was approved by the human ethics committee of Hasanuddin University, Makassar (No. 52/KEP/IV/2002).

Sensorineural deafness was diagnosed by audiologic testing with a pure-tone audiometer (Rion Audiometer AA-72B, Tokyo, Japan). The severity of hearing loss was classified according to the better ear: mild (27–40 dB), moderate (41–55 dB), moderate–severe (56–70 dB), severe (71–90 dB) and profound hearing loss (above 90 dB).

The A1555G (and also the A3243G) mutations were detected by PCR-RFLP as previously described (Lertrit et al. 1992; Malik

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et al. 2003). For sequencing of the hypervariable region 1 (HVR 1) of the mtDNA D-loop, the relevant fragment was PCR amplified using a primer pair, L15904 (5′-CTAATACACCAGTCTTG TAAA CCGGAG-3′) – H504 (5′-ATGGGCGGGGGTTGT ATTGATGAG-3′). Purified PCR products were sequenced with L15904, L16204 (5′-GCAAGTACAGCAATCAA CCCTC-3′) and H181 (5′-TAATATTGAACGTAGGTGCG-3′) as sequencing primers (Malik et al. 2002).

Results

Of the 75 patients from group I (non-syndromic sensorineural deafness), two (2.7%) had moderate, two (2.7%) moderate–severe, 18 (24%) severe, and 53 (70.6%) profound hearing loss. In comparison, 40 of the 50 patients with diabetes mellitus and sensorineural deafness (group II; 80%) had mild, five (10%) moderate, and five (10%) moderate–severe hearing loss. All the 100 control individuals were confirmed to have normal hearing. Four (5.3%) of the deafness patients from group I, but none from group II, were found to carry the mtDNA A1555G mutation (Fig. 1). This mutation was not found in the 100 control individuals.

We have tested at the same time for another mtDNA mutation, A3243G, in the $tRNA^{Leu}$ gene, which has been reported to be associated also with sensorineural deafness in significant prevalence, especially in syndromic cases with DM (Kadowaki et al. 1994; Nagata et al. 2001). However, this mutation was not found in the total of 125 sensorineural deafness patients (groups I and II) examined or in the control group.

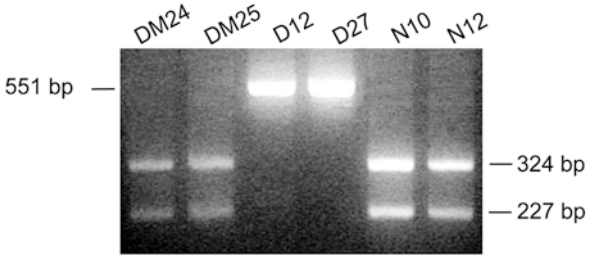
We are interested to find out whether the A1555G mutation in the four deafness patients had arisen independently, and have thus sequenced the HVR I of the mtDNA D-loop in these patients. Comparison of the sequencing data revealed that the four deaf patients were not related (Table 1).

Aminoglycosides are broad-spectrum antibiotics, many members of which (such as streptomycin) are still widely used in Asia, because they are relatively inexpensive. Local health authorities confirmed that streptomycin, as well as kanamycin and neomycin, have been widely used in South Sulawesi, but we could not ascertain whether the four mitochondrial sensorineural deafness patients found have been exposed previously to these antibiotics.

Discussion

Our finding has important medical implications. The absence of the mtDNA A3243G mutation in our patients, in particular in those who also had diabetes mellitus, is of significance and consistent with the result of our recent study. Of more than 1,500 Indonesian patients with Type 2 diabetes examined for this mutation, none were found to carry the A3243G mutation (Sudoyo et al. 2003), in contrast to around 0.9–2.8% in Japanese subjects (Kadowaki et al. 1994; Otabe et al. 1994; Kishimoto et al. 1995), 2.5% in Chinese subjects (Ng et al. 2000), and 0.2% in Korean diabetic patients

Fig. 1 Detection of mtDNA A1555G and A3243G mutations. The A1555G mutation was diagnosed by PCR-RFLP as described by Malik et al. (2003). The PCR amplification of a 551-bp mtDNA fragment was carried out using the primer pair L1231 and H1782. The A1555G base substitution was detected as a loss of a *Bsm*AI restriction site, and confirmed by sequencing using L1231 as the sequencing primer (Malik et al. 2003). Shown on the *left panel* are RFLP analyses of the PCR-amplified fragments from two sensorineural deafness patients with DM (DM 24 and DM 25), two sensorineural deafness patients showing the mtDNA A1555G mutation (homoplasmic; D12 and D27), and two normal controls (N10 and N12). The A3243G mutation was detected by a similar PCR-RFLP procedure, involving the amplification of a 903-bp fragment of the mtDNA with the primer pair L2826 and H3728, and the detection of the mutation as a gain of an *Apa*I restriction site (Lertrit et al. 1992)



	Deafness (n=75)	Deafness with DM (n=50)	Control (n=100)
A1555G	4	0	0
A3243G	0	0	0

Table 1 SNPs in the mtDNA of the deafness patients from Makassar carrying the A1555G mutation

rCRS	73A	89T	16108C	16126T	16144T	16148C	16162A	16183A	16189T	16258A	16265A	16274G	16304T	16311T	16362T	16519T
D12	G	C	-	G	C	T	C	C	C	-	C	-	-	C	-	-
D27	G	-	-	-	-	-	-	-	-	-	C	-	-	C	-	-
D65	G	C	T	-	-	-	G	C	C	-	-	A	-	C	C	C
D75	G	-	-	-	C	T	-	-	-	C	-	-	C	-	-	C

rCRS = revised Cambridge reference sequence (Anderson et al, 1989; Andrews et al, 1999)- D12, D27, D65 and D75 are sensorineural deafness patients carrying the mtDNA A1555G mutation

(Lee et al. 1997). The prevalence of this mutation in sensorineural deafness patients in Japan is 1.7% (Nagata et al. 2001), and as high as 50–60% of diabetic patients with sensorineural deafness (Kadowaki et al. 1994; Nagata et al. 2001). Thus, it seems that there is an ethnic-related variation in the prevalence of this mutation, presumably influenced by the overall mitochondrial genetic background.

The relatively high frequency of the A1555G mutation in the Indonesian sensorineural deafness patients indeed suggests that this mutation is an important contributor to sensorineural deafness in this region. The prevalence of the A1555G mutation in group I (non-syndromic sensorineural deafness) was 5.3%. The mutation was not found in the sensorineural deafness patients with diabetes mellitus, the group which has been included in this study for the specific purpose of examining the prevalence of the A3243G mutation. The overall prevalence of the A1555G mutation amongst the sensorineural deafness patients (a total of 125) was 3.2%, but the appropriateness of the diabetic group as part of the analysis of the prevalence of the A1555G mutation amongst the sensorineural deafness patients is uncertain.

The relationship of the phenotypic expression of this mutation and the use of aminoglycoside antibiotics is well established. Between 33% and 59% of sensorineural deafness patients with histories of exposure to aminoglycosides in Japan carry this mutation (Usami et al. 2000). Thus, should a screening for the mitochondrial DNA A1555G mutation be conducted before the administration of aminoglycoside antibiotics? The population prevalence for the A1555G mutation is not known. The average prevalence of deafness in 34 developing nations is estimated to be 1.4 hearing impaired per 1,000 (Mencher 2000). Taking 3–5.3% frequency of the A1555G mutation in Japanese and Indonesian sensorineural deafness patients (Usami et al. 2000 and this study), the population prevalence of the mutation would be around 1 per 13,500 to 1 per 24,000. In phenylketonuria, the clinical outcome of which can be completely ameliorated by early nutritional intervention, newborn screening is recommended on the basis of the incidence of 1 per 13,500–19,000 newborns (NIH Consensus Statement, 2000). By comparison, therefore, the screening for A1555G mutation before the administration of aminoglycosides should be considered.

Indeed in special cases, such a recommendation could be strongly advocated, e.g., when aminoglycosides have to be used for prolonged treatment. A particular example is the use of streptomycin in the treatment of tuberculosis. Despite its ototoxicity, this aminoglycoside antibiotic is still one of the first-line (essential) anti-tuberculosis drugs recommended by WHO (2003). In addition, kanamycin and amikacin are used as reserve (second-line) anti-tuberculosis (WHO, 2003). It is essential that further study is conducted to confirm the importance of this mutation as the cause of sensorineural deafness in Asia and elsewhere.

Acknowledgements We thank all the patients that have participated in the present study. The Eijkman Institute is supported by a development grant from the Ministry of Research and Technology of the Republic of Indonesia.

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