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ORIGINAL ARTICLE

Prevalence of mitochondrial DNA mutations in sporadic patients with nonsyndromic sensorineural hearing loss[☆]



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KEYWORDS

Mitochondria;
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Abstract

Introduction: Several mitochondrial DNA mutations have been reported to be associated with nonsyndromic hearing loss in several families. However, little is known about the prevalence of these mutations in sporadic patients with nonsyndromic sensorineural hearing loss.

Objective: The purpose of our study was to investigate the incidence of these mitochondrial DNA mutations in such population.

Methods: A total of 178 sporadic patients with nonsyndromic sensorineural hearing loss were enrolled in this study. Genomic DNA was extracted from the peripheral blood sample. We employed the SNaPshot[®] sequencing method to detect five mitochondrial DNA mutations, including A1555G and A827G in 12S rRNA gene and A7445G, 7472insC, and T7511C in tRNA^{Ser(UCN)} gene. Meanwhile, we used polymerase chain reaction and sequenced the products to screen GJB2 gene mutations in patients carrying mitochondrial DNA mutations.

Results: We failed to detect the presence of A1555G mutation in 12S rRNA gene, and of A7445G, 7472insC, T7511C mutations in tRNA^{Ser(UCN)} gene in our population. However, we found that 6 patients (3.37%) were carriers of a homozygous A827G mutation and one of them also carried homozygous GJB2 235delC mutation.

Conclusion: Our findings in the present study indicate that even in sporadic patients with nonsyndromic sensorineural hearing loss, mitochondrial DNA mutations might also contribute to the clinical phenotype.

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PALAVRAS-CHAVE

Mitocôndrias;
rRNA;
tRNA;
Deficiência auditiva

Prevalência de mutações no DNA mitocondrial em pacientes esporádicos com deficiência auditiva sensorioneural não síndrômica

Resumo

Introdução: Diversas mutações do DNA mitocondrial tem sido descritas, em diferentes famílias, associadas à deficiência auditiva não síndrômica. No entanto, pouco se sabe sobre a prevalência dessas mutações em pacientes esporádicos com deficiência auditiva sensorioneural não síndrômica.

Objetivo: A finalidade do nosso estudo foi investigar a incidência dessas mutações no DNA mitocondrial nessa população.

Método: No total, 178 pacientes esporádicos com deficiência auditiva sensorioneural não síndrômica foram recrutados para participação no estudo. O DNA genômico foi extraído de amostra de sangue periférico. Utilizamos o método de sequenciamento SNaPshot® para detecção de cinco mutações do DNA mitocondrial: A1555G e A827G no gene 12S rRNA e A7445G, 7472insC e T7511C no gene tRNA^{Ser(UCN)}. Paralelamente, utilizamos a reação de polimerase em cadeia e sequenciamos os produtos para triagem das mutações no gene GJB2 nos pacientes portadores de mutações no DNA mitocondrial.

Resultados: Em nossa população, não conseguimos detectar a presença da mutação A1555G no gene 12S rRNA e nem as mutações A7445G, 7472insC e T7511C no gene tRNA^{Ser(UCN)}. Entretanto, constatamos que seis pacientes (3,37%) eram portadores da mutação homozigota A827G; e um deles também portava a mutação homozigota GJB2 235delC.

Conclusão: Nossos achados no presente estudo indicam que, mesmo em pacientes esporádicos com deficiência auditiva sensorioneural não síndrômica, as mutações do DNA mitocondrial também podem contribuir para o fenótipo clínico.

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Introduction

Sensorineural hearing loss (SNHL) is one of the most common congenital disorders. The incidence is approximately one in 1000 newborns worldwide.¹⁻³ Over half of them have a genetic cause with autosomal dominant, autosomal recessive, X-linked or mitochondrial pattern of inheritance.^{4,5} Up to now, more than 200 point mutations in mitochondrial DNA (mtDNA) have been reported in the mtDNA mutation database MITOMAP.⁶ Among them, several mutations have been found to be associated with syndromic, nonsyndromic and aminoglycoside-induced hearing loss, especially those in 12S rRNA and tRNA^{Ser(UCN)} genes. The A1555G, which is located in the highly conserved A-site of mitochondrial 12S rRNA, was the first one to be described to be associated with SNHL and found to be responsible for both aminoglycoside-induced and nonsyndromic hearing loss in several families worldwide.⁷⁻¹⁰ The A827G, a homoplasmic mutation in mtDNA 12S rRNA, was recently found to be responsible for nonsyndromic hearing loss in all maternally related family members in a Chinese family.¹¹ It also has been reported to be associated with both aminoglycoside-ototoxicity and nonsyndromic hearing loss in sporadic individuals.^{12,13} Besides the 12S rRNA gene, the mitochondrial tRNA^{Ser(UCN)} gene is also associated with SNHL, as well as with nonsyndromic deafness, as several mutations have been identified, including A7445G,^{14,15} 7472insC,¹⁶ T7511C.^{17,18}

Although, as mentioned above, mtDNA mutations have been reported to be associated with nonsyndromic SNHL

in several families, little is known about the incidence of these mtDNA mutations in sporadic patients with nonsyndromic SNHL. Our previous study was focused on the GJB2 and SLC26A4 mutations in patients with autosomal recessive nonsyndromic hearing loss (ARNSHL). Consequently, we find that although the GJB2 and SLC26A4 gene are the most common deafness genes, only a small part of these patients carry homozygous or compound heterozygous pathogenic mutations.¹⁹ That indicates that other deafness genes may contribute to the clinical phenotype. In this study we used a rapid method, SNaPshot® sequencing, to screen 178 sporadic patients with nonsyndromic SNHL to estimate the prevalence of mtDNA mutations in such population. Moreover, to identify the role of the GJB2 gene in the deafness phenotype, we also screened the GJB2 gene in the patients carrying mtDNA mutations.

Methods**Patients and samples**

A total of 178 sporadic patients with nonsyndromic SNHL participated in this investigation. They originated from various regions of our province. Each patient received careful physical examination, and a comprehensive clinical history was recorded. Patients with dominant family history, a history of aminoglycoside exposure, and syndromic hearing loss were excluded from the study. All subjects underwent audiometric testing including auditory brain-stem

Table 1 PCR primer sequences.

Gene	PCR primers	Predicted size
12S rRNA		
A1555G	F: 5'-GCATCAAGCACGCAGCAATG-3'	926 bp
A827G	R: 5'-TAGGTTTAGCTCAGAGCGGTCAAGTTA-3'	
tRNA^{Ser}(UCN)		
A7445G	F: 5'-CCCCACCCTACCACACATTC-3'	524 bp
7472insC T7511C	R: 5'-GGTGTACTCGTAGGTTTCAAGTACCATTGG-3'	
GJB2		
Exon 1	F: 5'-TGGGGAAGTCATGGGGGCTCAAAG-3' R: 5'-AGGTTCTGGCCGGGCAGTCC-3'	425 bp
Exon 2	F: 5'-TCAGAGAAGTCTCCCTGTTCTGTCC-3' R: 5'-TGAGGCCTACAGGGGTTTCAA-3'	916 bp

Table 2 Extension primer sequences.

Gene	Extension primers
12S rRNA	
A1555G	R: 5'-TTTTTTTTTTTTTAACCCCTACGCATTATATAGAGGAG-3'
A827G	R: 5'-TTTTTTTTTTTACGGGAAACAGCAGTGATTA-3'
tRNA^{Ser}(UCN)	
A7445G	5'-TTTTTTTTTTTTTTTTTTTTTATTTCGAAGAACCCGTATACATAAAATCTAG-3'
7472insC	F: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAAGGAATCGAACCCCCCA-3'
T7511C	F: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCATGGCCTCCATGACTT-3'

response (ABR), otoacoustic emissions (OAE) and tympanometry. The severity of hearing impairment was defined as normal (<26 dB), mild (26–40 dB), moderate (41–70 dB), severe (71–90 dB) and profound (>90 dB). Peripheral blood samples were obtained from the patients for genomic DNA isolation.

Informed consent was obtained from adult patients and from the parents of children, and this study was approved by local Ethics Committee (Ethical Committee approval number: 2013–007).

SNaPshot® sequencing

A final 20 µL PCR reaction mixture contained 1× GC Buffer I, 3.0 mM Mg²⁺, 0.3 mM dNTPs, 1 U HotStarTaq polymerase, 10 ng template DNA, and 1 µM primer mixture, including two pairs of primers to amplify five mutation regions (Table 1). The PCR conditions were as follows: 95 °C for 2 min; 11 cycles of 94 °C for 20 s, 65–0.5 °C per cycle for 40 s, 72 °C for 90 s; 24 cycles of 94 °C for 20 s, 59 °C for 30 s, 72 °C for 90 s; 72 °C for 2 min. For purification of PCR products, 5 U shrimp alkaline phosphatase (SAP) (Promega) and 2 U Exonuclease I (Epicentre) were added into 15 µL PCR products. The mixture was incubated at 37 °C for 60 min, followed by incubation at 75 °C for 15 min.

The single base extension (SBE) was performed in a final 10 µL reaction mixture, containing 5 µL SNaPshot® Multiplex Kit (Applied Biosystems), 2 µL purified multiplex PCR product, and 0.8 µM extension primer mixture (Table 2). The

reaction program was 96 °C for 1 min; 28 cycles of 96 °C for 10 s, 52 °C for 5 s, 60 °C for 30 s; 4 °C forever. Then, the SBE products were purified by using SAP. For sequence analysis, 0.5 µL purified multiplex SBE products were mixed with 0.5 µL Liz120 SIZE STANDARD (Applied Biosystems) and 9 µL Hi-Di (Applied Biosystems), and denatured at 95 °C for 5 min. Then, the products were sequenced by the ABI 3730XL DNA sequencer. The data was analyzed by the GeneMapper v4.1 software (Applied Biosystems).

GJB2 mutation detection by polymerase chain reaction

Two exons of GJB2 were amplified from DNA samples by polymerase chain reaction (PCR). The primers were listed in Table 1. PCR amplification and subsequent purification were performed as we described before. The purified PCR products were sequenced using the dideoxy chain terminator method on an ABI 3730XL DNA sequencer (Applied Biosystems) with the ABI Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's protocols. The primers were the same as those for the PCR amplification.

Results

The study samples consisted of 82 males and 96 females. The age of all participants ranged from 9 months to 37

Table 3 Clinical and genetic characteristics of six patients carrying A827G in mtDNA 12S rRNA.

Patient	Sex	Age of onset (years)	Level of hearing impairment		A827G in mtDNA 12S rRNA	Mutations in GJB2
			Right	Left		
#61	Male	0	Profound	Profound	+/+	235delC/235delC
#105	Female	0	Profound	Profound	+/+	WT/WT
#131	Female	0	Profound	Profound	+/+	WT/WT
#140	Female	0	Profound	Profound	+/+	WT/WT
#197	Female	0	Profound	Profound	+/+	WT/WT
#210	Male	0	Profound	Profound	+/+	WT/WT

years, with a mean age of 5.53 ± 4.44 years. The results of audiometric testing revealed cochlear involvement in all participants and that all of them suffered from severe (71–90 dB to profound (>90 dB)) bilateral sensorineural hearing impairment.

In all 178 sporadic patients with nonsyndromic SNHL, we failed to detect the presence of the A7445G, 7472insC, T7511C mutations in the tRNA^{Ser(UCN)} gene. Moreover, we did not detect A1555G mutation in the 12S rRNA gene in this population. However, the A827G variant in the 12S rRNA gene was found in 6 patients, who all carried homozygous mutation. To illuminate the role of the GJB2 gene in the phenotypic expression in the individuals with mtDNA mutations, we screened the GJB2 gene mutations in the subjects carrying A827G mutation. Consequently, only one patient, who had homozygous 235delC, carried pathogenic mutation in the GJB2 gene (Table 3).

Discussion

Most of the previously reported studies focused on the association between the mtDNA mutations and the population with aminoglycoside-induced and/or nonsyndromic hearing loss.^{12,19} Several mtDNA mutations, such as A1555G and A827G in the 12S rRNA gene, A7445G, 7472insC and T7511C in the tRNA^{Ser(UCN)} gene have been identified to be associated with such populations.^{10,13,14,19,20} However, these mutations are often not sufficient to produce the clinical phenotype.^{14,19} In patients with ARNHL, similar with other studies, our previous study found that about 30–40% patients carried pathogenic mutations in the most common deafness genes, but nearly 40% of these patients carried heterozygous mutation. Thus, other gene mutations may together contribute to the phenotype. Although the association between the mtDNA mutations and nonsyndromic hearing loss has been identified in several families, little is known about the prevalence of mtDNA mutations in sporadic patients with nonsyndromic SNHL.

Mitochondrial DNA mutations in tRNA genes can cause tRNA modification and a failure in tRNA metabolism, thus impairing the protein synthesis and reducing the ATP synthesis, which are considered to be the main pathogenic factors.^{21–25} The A7445G mutation was first identified in a family with nonsyndromic deafness.²⁶ This mutation leads to the 3' end endonucleolytic processing defect in the L-strand polycistronic RNA precursor.²⁷ Consequently, the A7445G

mutation can cause a reduction of more than 50% in the tRNA^{Ser(UCN)} level and a decrease in protein synthesis, playing a determinant role in the respiratory phenotype of the mutant cell lines.^{28,29} The 7472insC was originally reported by Tiranti et al., which is likely to alter the structure of the T psi C loop in the tRNA^{Ser(UCN)} clover leaf secondary structure, and has been proved to impair both 5' and 3' processing of the tRNA^{Ser(UCN)} and cause a drop in the steady-state level of the tRNA^{Ser(UCN)}.^{16,24,30} The T7511C mutation has been identified to be associated with nonsyndromic hearing loss in several different ethnic families.^{17,18,31} This mutation can affect the processing of L-strand RNA precursor, spanning tRNA^{Ser(UCN)} as well as ND6 mRNA, specifically in the 5' end of tRNA.^{23,32} As a result, the T7511C mutation causes a reduction of the level of tRNA^{Ser(UCN)} and an impairment in mitochondrial protein synthesis.^{22,28} Although these mutations in the tRNA^{Ser(UCN)} have been found to be associated with the patients with aminoglycoside-induced and/or nonsyndromic hearing loss, many studies failed to detect such mutations in those patients. In the study reported by Xing et al., they analyzed the molecular characterization of a Chinese family with aminoglycoside-induced and nonsyndromic hearing loss, but did not find the A7445G, 7472insC, and T7511C mutations in the tRNA^{Ser(UCN)} gene.³³ In an Argentinean family with aminoglycoside-induced hearing loss, such mutations were also not found.³⁴ Moreover, Li et al. performed a molecular analysis in 164 unrelated Caucasian individuals with nonsyndromic hearing impairment and failed to detect the presence of the A7445G, 7472insC, and T7511C mutations in the tRNA^{Ser(UCN)} gene.¹² Abreu-Silva et al. also did not find these mutations in 203 unselected Brazilian hearing-impaired patients.³⁵ Up to now, little is known about the incidence of these mutations in the tRNA^{Ser(UCN)} gene in sporadic patients with nonsyndromic SNHL. In the present study, we explored these mutations in such population, but none of these subjects was found to carry such mutations in the tRNA^{Ser(UCN)} gene.

The A1555G mutation creates a specific GC base pair, which makes the secondary structure of mitochondrial 12S rRNA more closely resembling the corresponding region of *E. coli* 16S rRNA and binding aminoglycosides with high affinities.³⁶ Thus, the patients carrying this mutation can suffer from SNHL after using aminoglycosides.¹³ However, previous studies have identified that A1555G mutation in 12S rRNA is also associated with nonsyndromic hearing loss,^{9,10} while the incidence in nonsyndromic hearing loss is

much lower than in aminoglycoside-induced hearing impairment. Lu et al. reported that the incidences of the A1555G mutation were 1.43% and 10.41% in a Chinese pediatric population with nonsyndromic and aminoglycoside-induced hearing loss, respectively.¹⁹ Similarly, in a Polish population, Rydzanicz et al. observed that the incidence was 5.5% and 1.6% in the cohorts with aminoglycoside-induced and nonsyndromic hearing loss.³⁷ In a Caucasian pediatric nonsyndromic hearing loss population, the frequency of the A1555G mutation was 0.6%.¹² In this study, we found that none of the subjects carried the A1555G mutation. Compared with previous reports, the incidence of the A1555G mutation in our population with nonsyndromic SNHL is relatively lower.

The A827G mutation, like A1555G mutation, is located at the A-site of the mitochondrial 12S rRNA gene, which is highly evolutionarily conserved in different species.³⁴ It is possible that the alteration of the tertiary or quaternary structure of the 12S rRNA by the A827G mutation may lead to mitochondrial dysfunction; thereby, it would play a role in the pathogenesis of hearing loss.^{11,34} The pathogenesis of this mutation has been identified in a Chinese family with nonsyndromic hearing loss.¹¹ Although the A827G mutation was also found in normal hearing controls,¹⁹ more studies consider it as a pathogenic mutation depending on its location and absence in the controls in their studies.^{11–13,34,37} Meanwhile, these studies also found incomplete penetrance of the A827G mutation, which indicates that this mutation alone is not sufficient to produce clinical phenotype.^{11,34} Thus, some normal hearing subjects carrying the A827G mutation may be attributed to incomplete penetrance. In our study, we found that 3.37% (6/178) patients carry homozygous A827G mutation. In another Chinese sporadic pediatric population with nonsyndromic hearing loss, the incidence is 4.41%.¹³ The incidence of the A827G mutation in such Chinese population seems to be higher than in other ethnic groups.^{11,37} As previous studies showed that expression of the clinical phenotype of deafness-associated homoplasmic mutations in the 12S rRNA gene requires the contribution of modulating factors, including aminoglycosides or nuclear modifier genes, and the GJB2 gene is a potential candidate modifier gene,^{12,38–40} we performed mutational screening of the GJB2 gene in the patients carrying the A827G mutation. As a result, only one patient carried both 12S rRNA A827G mutation and homozygous GJB2 235delC mutation. The lack of GJB2 mutation in other patients indicates that other nuclear modifier genes and other environmental factors may contribute to the clinical phenotype in these patients.

Conclusion

In the present study we screened mtDNA mutations in sporadic patients with nonsyndromic SNHL and found a total of 6 subjects (3.37%) carrying homozygous mtDNA mutations. Our findings indicate that even sporadic patients with nonsyndromic SNHL also carry mtDNA mutations and these mutations might contribute to the clinical phenotype. Further studies screening the mutations in mtDNA are needed to help us understand the prevalence and role of mtDNA mutations in sporadic patients with nonsyndromic SNHL.

Conflicts of interest

The authors declare no conflicts of interest.

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