GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 16, Number 9, 2012

Pp. 1146–1148
DOI: 10.1089/gtmb.2011.0365

Mitochondrial DNA Mutation Screening in an Ethnically Diverse Nonsyndromic Deafness Cohort

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Deafness is a heterogeneous trait with many known genetic and environmental causes. Hereditary hearing loss is an extremely common disorder in the general population. Mutations in mitochondrial DNA (mtDNA) are known to be associated with nonsyndromic deafness (NSD) and syndromic deafness. The objective of this article is to investigate the frequency of common mitochondrial mutations (A1555G, G7444A, and A3243G) in an ethnically diverse cohort of probands with NSD from South Florida. These patients were ascertained at the University of Miami. Polymerase chain reaction–restriction fragment length polymorphism analysis and direct sequencing methods were used for mutation screening in a cohort of 217 patients with NSD. The frequency of common mitochondrial mutations is 1.84% (4/217) in this cohort. A1555G and G7444A accounted for four patients with NSD. Our mutation frequencies are comparable with those previously reported in other populations, indicating that mutations in mtDNA are an important cause of NSD in our patient cohort.

Introduction

Hearing loss (HL) is a common congenital disorder and is an economically and socially important cause of human morbidity. Deafness can result from a mutation in a single gene or from a combination of mutations in different genes; environmental causes, medical disorders such as otitis media and tinnitus, environment exposure, trauma, and medications, or interactions between genetic and environmental factors. Worldwide, congenital deafness occurs in ~1 in 1000 live births, and more than half of these cases are hereditary (Morton, 1991). Sensorineural hearing loss (SNHL) is present in 42% to 70% of individuals with mitochondrial disorders and can be syndromic or nonsyndromic (Ouyang *et al.*, 2009).

Multiple genes are currently under study for their role in mitochondrial-associated hearing impairment. The mitochondrial $tRNA^{Ser\ (UCN)}$ (*MTTS1*) gene appears to be a significant mitochondrial DNA (mtDNA) mutation locus associated with nonsyndromic sensorineural hearing loss (NSHL). Four deafness-associated mutations, A7445G, 7472insC, T7510C, and T7511C, have been identified in this gene (Ouyang *et al.*, 2009).

Additionally, mitochondrial *12S rRNA* (*MTRNR1*) is another significant locus for nonsyndromic mtDNA mutations. The homoplasmic A1555G mutation of this ribosomal RNA (rRNA) has been associated with aminoglycoside-induced and NSHL in many families of different ethnicities (Ouyang *et al.*, 2009).

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) are typically associated with a point mutation at nucleotide 3243 (A3243G) in the $tRNA^{Leu(UUR)}$ (*MTTL1*) (Goto *et al.*, 1990). The prevalence of this mutation has been estimated to range from 0.5% to 60%, depending on the subgroup of diabetics under study and ethnic background (Chinnery and Turnbull, 2001).

The objective of this study is to determine the frequency of mutations in three common mtDNA mutations, A1555G, A7445G, and A3243G, in an ethnically diverse NSHL cohort.

Materials and Methods

All subjects participating in the study were from the University of Miami Ear Institute. The cohort of probands was recruited from the outpatient service between 2001 and 2010. This study was open to all patients with a diagnosis of mild or greater SNHL, and only individuals with nonsyndromic deafness (NSD) were included in this report. Following the Institutional Review Bboard approval from the University of Miami, 217 patients with nonsyndromic sensorineural hearing impairment were recruited. The age of the patients varied between 3 months and 80 years, with a mean age of 22.7, a

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median of 10 and a mode of 4. Information on the medical history and pedigree structure was obtained through personal interviews with the affected individuals or with their unaffected relatives. Written informed consent was obtained from all adult participants and from parents of patients younger than 18 years.

Genomic DNA was extracted from peripheral blood using a standard extraction method and prescreened for mutations in the coding and noncoding exons of the *GJB2* gene and in del *GJB6-D13S1830*. After exclusion of these two genes as potential causes of HL, a total of 217 of patients were included in this study for a molecular etiology analysis of deafness by screening the common mtDNA mutations.

Analysis of mtDNA A1555G, A3243G, and G7444A mutations was performed as previously described (Pandya *et al.*, 1999; Kabahuma *et al.*, 2011). Bidirectional sequencing was carried out using the ABI PRISM Big Dye Terminator Sequencing kit (Applied Biosystems) on a 3100 ABI DNA-sequencer (Applied Biosystems).

Results and Discussion

The 217 probands screened for mtDNA mutation were classified based on their self-reported race. A total of 117 (53.9%) were whites of European ancestry, 16 (7.4%) were African American, 70 (32.3%) were whites Hispanic/Latino, 11 (5.1%) were Asian, 2 (0.9%) were of Middle Eastern descent, including 1 Palestinian, 1 Israeli Arab, and 1 Portuguese (0.46%) (Table 1). Of the total probands screened, four (1.84%, 4/217) were noted to have mtDNA mutations. Two probands were found to carry the A1555G (0.9%, 2/217) and two had the G7444A (0.9%, 2/211) mutation. Both variants were found in a homoplasmic state.

The A1555G mtDNA in 12S rRNA was the first mtDNA mutation identified as a cause of maternally inherited nonsyndromic HL, and it has been implicated in aminoglycoside-associated HL (Pandya *et al.*, 1997). The prevalence of the A1555G mutation varies in nonsyndromic hearing-impaired populations by ethnicity: 2.9%–5.3% in Asian patients; 0.6%–2.5% in Caucasian deaf populations (Ouyang *et al.*, 2009). In our study, the 1555A>G mutation was found in 0.9% (2/217) patients as a homoplasmic mutation, and at least one patient had a significant history of ototoxic medication exposure. A previous review of A1555G by Usami *et al.* (2000), noted an incidence of 3% in all patients with SNHL and a 33% incidence in patients with aminoglycoside exposure. The first patient with the A1555G mutation was an 80-year-old white female with progressive HL that presented at approximately age 40. This patient had no known history of aminoglycoside exposure. She was implanted with a Nucleus 24 cochlear implant (Cochlear Corp.) in the right ear. Postoperative hearing in a noise test result was 73% on average. The second patient, a Hispanic female in her 5th decade with progressive HL was noted to have a history of aminoglycoside exposure as a child and a clinical history suggestive of endolympathic hydrops. The patient was prescribed hearing amplification and treated with hydrochlorothiazide/triamterene 25/37.5 mg PO daily for hydrops and Coenzyme Q 10 150 mg daily for the mtDNA mutation. She responded well to both diuretic and antioxidant therapy.

The G7444A substitution has been described in deaf individuals with and without the A1555G mutation. Pandya *et al.* (1999) reported that the G7444A variant may have influenced the severity and age at onset of HL associated with the A1555G mutation in Mongolians, secondary to its effect on the oxidative phosphorylation cycle. The pathogenicity of the G7444A variant is still not clear. The involvement of the G7444A substitution in the pathogenesis of HL is mostly based on Chinese patients studies (Zhu *et al.*, 2006; Jin *et al.*, 2007; Yuan *et al.*, 2007). The analysis of the G7444A substitution in the $tRNA^{Ser(UCN)}$ gene in Caucasian patients has not confirmed the implication of this mutation in NSD (Leveque *et al.*, 2007; Li *et al.*, 2007). The relatively high carrier frequency of $tRNA^{Ser(UCN)}$ G7444A (1/62) in the Polish population (Rydzanicz *et al.*, 2009) and its prevalence of about 1% (2/190) in individuals of African ancestry suggest that it is probably a polymorphism (Abreu-Silva *et al.*, 2006). G7444A is a common variant within the West European prevalent haplogroup V (Kivisild *et al.*, 2006). Moreover, G7444A has also been found in two H sequences (Abreu-Silva *et al.*, 2006), and one L1b sequence (Kivisild *et al.*, 2006). These findings further support that this substitution constitutes a normal polymorphism.

The two patients identified in our study with G7444A are whites and had adult onset moderate HL. Patient 1 noted a significant family history suspicious for mitochondrial inheritance, whereas patient 2 noted a sporadic history, which may suggest a *de novo* mutation. Our assays did not reveal a secondary mutation, which may have potentiated the effects of the G7444A mutation. Both individuals were treated with Coenzyme Q 10 with no noted progression of their disease. Previous work from our group has demonstrated the preliminary data suggesting the efficacy of Coenzyme Q10 in these same patients (Angeli *et al.*, 2005). Coenzyme Q10, an antioxidant, has been shown to inhibit lipid peroxidation by removal of reactive oxygen species. Recent studies on the effect of coenzyme Q10 on noise-induced HL with guinea pigs have noted a significant protective effect on hair cells with preservation of hearing (Fetoni *et al.*, 2009). Further studies are necessary to fully characterize the efficacy of this treatment modality.

In conclusion, the incidence of mtDNA mutations in the cohort of screened patients was 1.8% (4/217). The frequency of A1555G and G7444A is 0.9% (2/217). Of the four mtDNA mutations found by screening of 217 individuals in the present study, 3 were identified in whites (3/117; 2.6%) and 1 in an individual of Hispanic/Latino ethnicity (1/70; 1.4%).

Table 1. Demographic Data

		Gender		Race				
Age	*Age at onset*	*Female*	*Male*	*White, nonHispanics*	*White, Hispanics*	*African-American*	*Asian*	*Other*
3 month to 80 years	Birth to 79 years	113	104	117	70	16	11	3

Although mtDNA mutations at higher than expected rates have been reported in some Asian populations, none of the 11 Asian subjects (a mean age of 13 and a median of 3) tested carried mtDNA mutations. The unregulated use of ototoxic drugs remains a major problem in developing countries and aminoglycoside ototoxicity accounts for 3% to 30% of HL. This high prevalence rate may be associated with genetic susceptibility through mtDNA mutations. Overall, our data suggest that mtDNA mutations are one of the important etiologies of HL in patients with NSD in South Florida. They also show that the connection of mtDNA mutations to clinical phenomenon impacts a clinician's ability to diagnose, treat, and counsel patients effectively, and that therapies can be tailored to each individual's unique problem.

Acknowledgment

This work was supported by NIH grant DCR01 05575.

Author Disclosure Statement

No competing financial interests exist.

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