Prevalence of the A1555G (12S rRNA) and tRNA[Ser(UCN)] mitochondrial mutations in hearing-impaired Brazilian patients

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Abstract

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Mitochondrial mutations are responsible for at least 1% of the cases of hereditary deafness, but the contribution of each mutation has not yet been defined in African-derived or native American genetic backgrounds. A total of 203 unselected hearing-impaired patients were screened for the presence of the mitochondrial mutation A1555G in the 12S rRNA gene and mutations in the tRNA[Ser(UCN)] gene in order to assess their frequency in the ethnically admixed Brazilian population. We found four individuals with A1555G mutation (2%), which is a frequency similar to those reported for European-derived populations in unselected samples. On the other hand, complete sequencing of the tRNA[Ser(UCN)] did not reveal reported pathogenic substitutions, namely A7445G, 7472insC, T7510C, or T7511C. Instead, other rare substitutions were found such as T1291C, A7569G, and G7444A. To evaluate the significance of these findings, 110 "European-Brazilians" and 190 "African-Brazilians" unrelated hearing controls were screened. The T1291C, A7569G and G7444A substitutions were each found in about 1% (2/190) of individuals of African ancestry, suggesting that they are probably polymorphic. Our results indicate that screening for the A1555G mutation is recommended among all Brazilian deaf patients, while testing for mutations in the tRNA[Ser(UCN)] gene should be considered only when other frequent deafness-causing mutations have been excluded or in the presence of a maternal transmission pattern.

Introduction

Hereditary deafness is a heterogeneous condition. Approximately 80-85% of the cases of hereditary non-syndromic sensorineural hearing loss exhibit a recessive mode of inheritance, whereas 15% have a dominant transmission; X-linked and mitochondrial inherited cases are considered to add up to about 1% (1).

The first mitochondrial mutation described in non-syndromic sensorineural hearing loss was the A1555G substitution in the 12S ribosomal RNA gene (*MT-RNR1*). This mutation causes hypersensitivity to aminoglycosides (2,3) since it alters 12S rRNA, making it more similar to the bacterial ribosomal subunit and thereby enhancing aminoglycoside binding and its toxic effects on the ear (3,4).

An outstanding survey was reported by Estivill et al. (5), who studied 19 Spanish families with sensorineural deafness due to the mtDNA A1555G mutation. The authors determined age-dependent penetrance values of 50% by the age of 30 years and of 88% by the age of 65 years. They showed that the penetrance could be enhanced by aminoglycosides, so that the probability of deafness at 30 years of age was 96.5% in the treated group and only 39.9% in the untreated group.

Four mtDNA mutations have been found to affect the tRNA$^{Ser(UCN)}$ gene (*MT-TS1*), i.e., A7445G, 7472insC, T7510C, and T7511C, causing deafness. Reid et al. (6) first described the A-to-G transition at position 7445 in a Scottish family. Tiranti et al. (7) reported a large kindred from Sicily with a new maternally inherited syndrome characterized by hearing loss, ataxia, and myoclonus. mtDNA sequencing revealed an insertion of a cytosine at nucleotide position 7472 (7472insC). Sue et al. (8) first described a T7511C point mutation in a large African American family with maternally transmitted, symmetric and gradually progressive hearing loss. T7510C was the most recently described mutation in the tRNA$^{Ser(UCN)}$ gene (9).

Pandya et al. (10), investigating a sample of 480 deaf students in Mongolia, identified by restriction analysis nine individuals with results suggesting the A7445G mutation. However, mtDNA sequencing revealed that in fact two of them had the A7445G substitution and one had an A7443G substitution. The other six had a G7444A substitution. Surprisingly, the six patients with the G7444A substitution also had the A1555G substitution. This was the first report of the A7443G and G7444A substitutions associated with hearing loss.

The Brazilian population represents a complex admixture of ethnic groups. South American Indians, Portuguese settlers, and African slaves contributed to its gene pool, and during the last hundred years the country received immigrants from Europe, Asia and the Middle East. Alves-Silva et al. (11) investigated 247 Brazilian individuals mainly classified as "white" from five geographic regions, and found almost equal amounts of native American, African and European mtDNA. This admixture probably has a bearing on the frequency of disease-associated mtDNA mutations.

We report here an investigation of the contribution of mitochondrial mutations to hearing impairment in a sample of the Brazilian population.

Subjects and Methods

Subjects

Individuals affected by hearing loss were identified at our genetic counseling unit, Centro de Estudos do Genoma Humano, State of São Paulo. They had been referred to us by the following institutions: DERDIC (Divisão de Educação e Reabilitação de Distúrbios da Comunicação, Pontifícia Universidade Católica de São Paulo), CEPRO (Centro de Ensino Profissionalizante Rotary), Hospital das Clínicas, Faculdade de Medicina, USP, all in the State of São Paulo, and Universidade Estadual de Maringá, State of Paraná.

Family 1 was selected because of maternal inheritance and possible aminoglycoside exposure. The other 203 individuals studied formed an unselected series of hearing-impaired subjects: 136 were isolated cases, 64 had at least one affected relative, and three had an unknown history. The onset of de

afness was prelingual in 151 subjects, post-lingual in 42, and 10 were unclassified. The degree of hearing impairment was profound in 69% of cases, severe in 15%, moderate in 15%, and mild in 1%. Most patients (62%) were classified as "white", 36% were classified as African-Brazilians and 2% were of Asian origin (Japanese or Chinese).

The control sample was formed by 190 African-Brazilians and 110 European-Brazilians without hearing impairment.

The research plan was approved by the Ethics Committee of the Instituto de Ciências Biomédicas, Universidade de São Paulo. Written informed consent for analysis was obtained from all normal controls, patients, and their relatives participating in the study.

Methods

DNA was extracted from whole blood employing standard phenol/chloroform techniques as described by Kunkel et al. (12). All patients were screened for the 35delG mutation (*GJB2* gene) by allele-specific PCR according to Scott et al. (13). All positive cases, heterozygotes or homozygotes, were confirmed by sequencing the *GJB2* gene. The A1555G mutation was screened according to the protocol of Estivill et al. (5). PCR amplification and restriction analysis (*Hae*III digestion) detect both the A1555G mutation and the T1291C substitution. PCR products of the wild-type *MT-RNR1* gene yield two restriction fragments (216 and 123 bp after *Hae*III digestion), PCR products of individuals carrying the A1555G mutation yield three restriction fragments (216, 93, and 30 bp), while PCR products of individuals carrying the T1291C substitution yield three fragments (173, 123, and 43 bp). All PCR products that deviated from the wild-type pattern were sequenced with the same set of primers.

Mutations A7445G, 7472insC, T7510C, T7511C in the *MT-TS1* gene and others affecting base pairs 7392 to 7608 were inves-tigated by mtDNA sequencing with primers 5'GGA TGC CCC CCA CCC TAC C3' and 5'CCT ACT TGC GCT GCA TGT GCC3' (9), using the DYEnamic ET Dye Termina-tor Cycle Sequencing Kit (Amersham Biosciences, Buckinghamshire, UK) and analyzed with the MegaBACE 1000 DNA Analysis System (Amersham Biosciences).

The 300 normal controls were screened by restriction site analysis for the A1555G and T1291C (*Hae*III), G7444A and A7445G (*Xba*I), T7510C and A7569G (*Hin*fI) substitutions.

All the individuals who presented mitochondrial mutations or rare substitutions were tested for the *Hpa*I restriction site at nucleotide 3592, which defines the African macro-haplogroup L1/L2 (14), and for the presence of the 9-bp deletion which defines the mitochondrial haplogroup B (15), probably of Amerindian origin in the Brazilian context.

Results

Table 1 summarizes the results of the screening for known pathogenic mutations and also of other rare substitutions (T1291C, A7569G, and G7444A). Given the unknown significance of these substitutions, they were screened among normal controls. The three substitutions were detected only among African-Brazilians (Table 1). Family 1 is not included in the Table.

The sequencing of the mtDNA region from nucleotides 7392 to 7608 in the hearing-impaired subjects also permitted the identification of previously known polymorphisms, which were not included in Table 1: A7424G in the cytochrome C oxidase subunit I gene (16) was found in 1.5% (3/203), C7498T in the tRNA[Ser(UCN)] gene (17) was found in 1% (2/203) and G7521A in tRNA[Asp] gene (16) was found in 25% (51/203) probands.

The five probands with A1555G (including the proband of Family 1), the five hearing-impaired carriers of T1291C, one of the

two normal carriers of T1291C and also the three (one deaf, two normal) carriers of the A7569G substitution belong to the macro-haplogroup L1/L2, indicating an African origin of the mtDNA. The affected proband with the G7444A mutation, as well as one of the normal carriers of G7444A presented the 9-bp deletion, indicating probable native American origin.

The five families presenting the A1555G mutation are described below:

Family 1 (Figure 1): Nine individuals affected by sensorineural nonsyndromic progressive postlingual hearing loss were studied, the onset of the defect occurring during adult life in 6 of them. The A1555G mutation was tested because the affected indi-

vidual with the earliest onset (III-1) presented deafness after antibiotic treatment (possibly aminoglycosides) and the mutation was detected in all 9 individuals. A1555G was also detected in two hearing individuals, IV-9, IV-11, and in patient II-8, who presented a mild hearing loss possibly due to presbyacousis.

Family 2: The only affected individual was ascertained at the age of 6 years, with congenital bilateral profound sensorineural deafness. His unaffected mother carried the same mutation.

Family 3 (Figure 2A): The proposita (IV-7) was born to a consanguineous couple. Ascertained at the age of 39, she presented postlingual bilateral profound sensorineural

Table 1. Frequency of pathogenic mutations and rare substitutions in mtDNA detected among hearing-impaired Brazilian subjects and normal Brazilian controls.

Subjects	N	Pathogenic mutations					Rare substitutions		
		A1555G	A7445G	7472insC	T7510C	T7511C	T1291C	G7444A	A7569G
Hearing-impaired	203	4 (2%)	0	0	0	0	5 (2%)	1 (0.5%)	1 (0.5%)
Normal controls									
African-Brazilians	190	0	0	–	0	–	2 (1%)	2 (1%)	2 (1%)
European-Brazilians	110	0	0	–	0	–	0	0	0

Figure 1. Pedigree of Family 1 with mtDNA A1555G mutation.
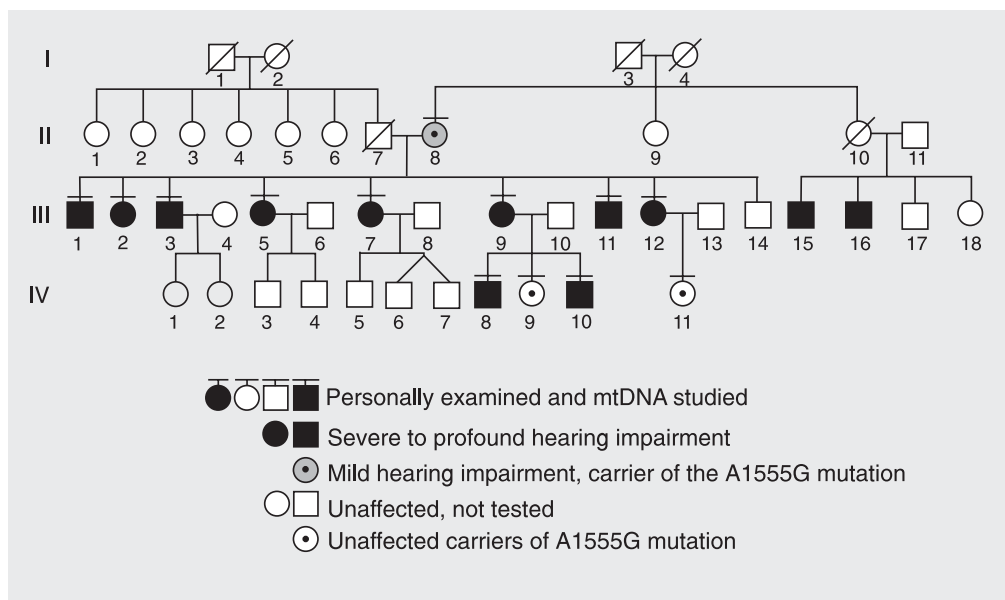
deafness. Two brothers (IV-4, IV-5) and a sister (IV-6) had congenital profound deafness. In another sister (IV-3), onset was postlingual. Both the A1555G and the T1291C substitutions were detected in the family (Figure 2B), but none was present in III-1, the proposita's father.

Family 4 (Figure 3): The proband (IV-3), born to a consanguineous couple and ascertained at the age of 7 years, had profound prelingual deafness, mental retardation, hyperactivity, and seizures. Mutation A1555G was detected in her father (III-5), in her mother (III-6) and in the 2 unaffected sisters (IV-1 and IV-2). All the other affected family members (including the father and the mother) presented a less severe hearing impairment than the proband.

Family 5: Ascertained at the age of 1 year 8 months, the patient presented bilateral sensorineural non-progressive profound hearing loss. Skin and hair pigmentation was visibly lighter than in the other members of the family. Her mother, grandmother and two sisters also carried the A1555G mutation. The mother presented high frequency hearing loss and speech difficulties. The two sisters had normal audiograms, but otoacoustic emissions with minor alterations indicated outer hair cell loss.

Discussion

The most common mitochondrial mutation associated with deafness is the A1555G substitution in the 12S rRNA gene, which has been detected in patients of different ethnic backgrounds. In Japan, for instance, approximately 3% of sensorineural deafness patients and 10% of cochlear implantation patients carry the A1555G mutation (18).

The present study showed a contribution of 2% of A1555G to non-syndromic sensorineural hearing loss in Brazilian probands. The majority of our 203 probands had prelingual deafness, a feature also found in some European studies (19-21) that reported

A1555G frequencies ranging from 0 and 2.4%. If 35delG homozygotes and heterozygotes (the most prevalent connexin 26 mutation) are excluded from our sample (N = 26), the proportion of A1555G is 4/177 (2.25%). Therefore, the A1555G mutation appears to be the underlying cause in about 2% of hearing loss cases of predominantly prelingual onset, and our results show that the Brazilian sample does not differ significantly from other reported series.

A1555G mutation was detected in 4.7% of 64 familial cases. The segregation pattern
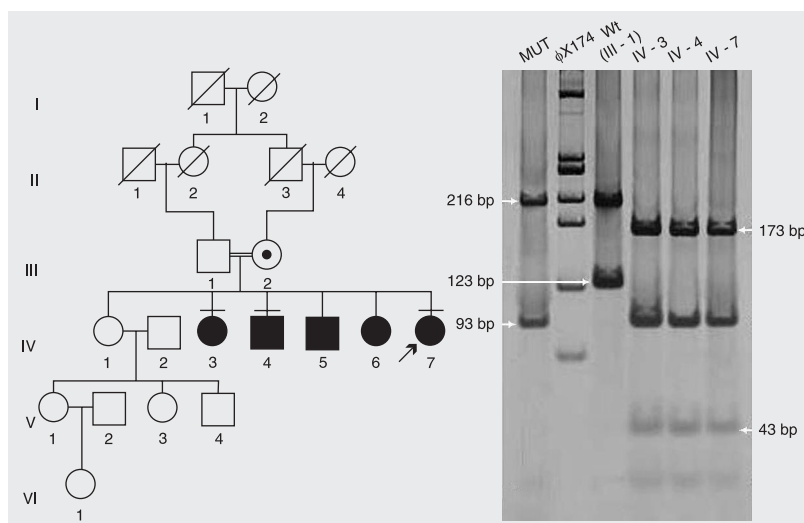

Figure 2. *A*, Pedigree of Family 3 with mtDNA A1555G and T1291C substitutions. Symbols are defined in Figure 1. *B*, Polyacrylamide gel electrophoresis (6%) with restriction patterns: MUT = the 216-, 93-bp fragments (the 30-bp fragment also found in A1555G carriers is not shown); WT (III-1) = the 216- and 123-bp bands from the wild-type father; IV-3, IV-4 and IV-7 = carriers of both A1555G and T1291C substitutions. The restriction fragments (173 and 43 bp) generated when the T1291C substitution is present are indicated by arrows on the right.
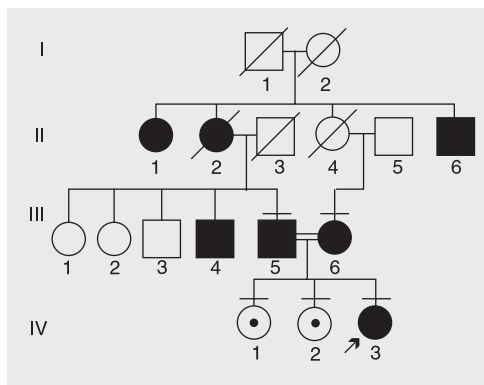

Figure 3. Pedigree of Family 4 with the mtDNA A1555G mutation. Symbols are defined in Figure 1.

of the defect in one of these families (Family 3, Figure 2) suggested autosomal recessive inheritance. In Family 2, the proband was an isolated case. If we had adopted maternal transmission or familial deafness as a selection criterion, the two families would not have been detected.

We identified three rare substitutions: T1291C, G7444A and A7569G. Both T1291C and G7444A substitutions have been reported one and three times, respectively, in a list of polymorphic sites in mtDB (Human Mitochondrial Genome Database) among the 1007 sequences recorded in this databank (22). On the other hand, the A7569G substitution was not found in any databank (mtDB - Human Mitochondrial Genome Database; GenBank; MITOMAP). In our control group of 300 subjects, 190 of them African derived, and 110 European derived, we found two T1291C, two A7569G and two G7444A carriers, all of them classified as African-Brazilians (Table 1). Therefore, these substitutions probably represent polymorphisms of African origin. The presence of the *Hpa*I restriction site at nucleotide 3592, which defines the African macrohaplogroup L1/L2 (14), in two normal A7569G carriers and one normal T1291C carrier supports this interpretation. The other patient with the rare G7444A substitution has mtDNA from haplogroup B (Amerindian).

The clinical presentation of deafness in A1555G probands and in their relatives varied in severity, progression, and age of onset, as previously reported (5). In all families reported here and in the literature, the phenotypes of carriers of the A1555G mutation ranged from normal hearing to profound congenital deafness. Aminoglycoside treatment was not reported by the probands. In one case in Family 1 (III-1) aminoglycoside treatment was probably responsible for the early onset of deafness. Thus, lack of penetrance and variable expressivity of deafness may result from other genetic or environmental factors.

The proband in Family 4 showed other clinical signs together with hearing loss. Her parents were first cousins (Figure 3) and it is possible that the accompanying clinical signs resulted from the homozygosity for deleterious genes. She was the most hearing-impaired subject among all family members. Surprisingly, in Family 3, the affected individuals, also born to a consanguineous couple, presented significantly more severe prelingual hearing loss than usually observed in A1555G carriers. Both families raise the possibility of modifier mutations with an autosomal recessive mode of inheritance, which could enhance the effects of the A1555G substitution. The influence of nuclear-encoded modifier genes has been suggested in the literature (23). Bykhovskaya et al. (24) suggested the presence of a putative modifier gene on 8p23.1, but its identification has remained elusive. The same group suggested that mitochondrial RNA modification is an important regulatory pathway in the phenotypic expression of the A1555G mutation. They provided evidence that three genes involved in the process of mitochondrial RNA modification, *TFB1M*, *MTO1* and *GTPBP3*, may be nuclear-encoded modifier genes of the phenotype associated with the A1555G mutation (25,26). However, no obviously pathogenic mutation has ever been identified in those genes and evidence was based only on linkage results.

Nye et al. (27) reported a Filipino-American family with the A1555G mutation in which hearing loss was associated with clinical signs suggestive of Waardenburg syndrome. The proband in our Family 5 presented lighter skin and hair pigmentation than other relatives. This raises the possibility that mitochondrial mutations associated with deafness also affect pigmentation, in a way similar to that observed for the many previously reported autosomal mutations.

The proband carrying the G7444A mutation had congenital heart defects, low weight,

prominent and low-set ears, triangular face, midface hypoplasia, antimongoloid palpebral slants, micrognathia, highly arched palate, and slender hands and fingers. Pandya et al. (10) reported association of the G7444A substitution with hearing loss, but always with A1555G. In another study (28), the G7444A substitution was found alone, not associated with A1555G, in a DNA repository of deaf probands in the United States. This mutation has also been reported in association with Leber hereditary optic neuropathy, and was considered to be a secondary change increasing the penetrance of the primary mutation, but insufficient *per se* to cause Leber hereditary optic neuropathy (29). Thus, the role of G7444A as a cause of deafness is not defined. Our screening of normal carriers in African-Brazilians indicates that it represents in fact a polymorphism. The possibility that it might enhance the severity of the clinical manifestation of another etiological factor is unlikely, given that its frequency among affected and controls was similar. Distinction between pathogenic mutations and population-specific polymorphic sites by adequate selection of normal controls is fundamental in genetic counseling.

Our findings suggest that the mitochondrial mutation A1555G is a common cause of hearing impairment in Brazil, as reported in other populations, and should be screened in the diagnosis of deafness. The identifica-tion of the A1555G mutation in isolated or familial cases of deafness, the presymptomatic detection of this mutation in maternally related subjects, the avoidance of aminoglycosides and other risk factors by individuals who are positive for the A1555G mutation, and genetic counseling should help in prevention of deafness. On the other hand, testing for the A7445G, 7472insC, T7510C, and T7511C mutations should be considered only in selected individuals. We suggest their screening only after A1555G and other common mutations (as 35delG in *GJB*2) have been excluded and when a pattern of maternal inheritance is present.

Acknowledgments

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