

# Genetic Basis of Nonsyndromic Sensorineural Hearing Loss in the Sub-Saharan African Island Population of São Tomé and Príncipe: The Role of the *DFNB1* Locus?

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## Abstract

Hearing loss (HL) is a common condition with both genetic and environmental causes, and it greatly impacts global health. The prevalence of HL is reportedly higher in developing countries such as the Sub-Saharan African island of São Tomé and Príncipe, where the deaf community is estimated to be less than 1% of the population. We investigated the role of the *DFNB1* locus (*GJB2* and *GJB6* genes) in the etiology of nonsyndromic sensorineural hearing loss (NSSLH) in São Tomé and Príncipe. A sample of 316 individuals, comprising 136 NSSLH patients (92 bilateral, 44 unilateral) and 180 controls, underwent a clinical and audiological examination. Sequencing of the *GJB2* coding region and testing for the (*GJB6*-D13S1830) and del(*GJB6*-D13S1854) *GJB6* deletions were performed. A total of 311 out of 316 individuals were successfully analyzed regarding the *GJB2* and *GJB6* genetic variations, respectively. The frequency of the *GJB2* coding mutations in patients and controls was low. Some of those coding mutations are the most commonly found in Eurasian and Mediterranean populations and have also been identified in Portugal. None of the *GJB6* deletions was present. The presence of certain coding variants in São Tomé and Príncipe suggests a non-Sub-Saharan genetic influx and supports the previously reported genetic influx from European (mainly Portuguese) ancestors. In summary, *DFNB1* locus does not appear to be a major contributor to NSSLH in São Tomé and Príncipe. However, the presence of both pathogenic and likely pathogenic mutations in *GJB2* suggests that *GJB2*-related NSSLH might still occur in this population, warranting further research on *GJB2* testing in NSSLH cases.

## Introduction

**H**EARING LOSS (HL) is a condition that is an outcome of both environmental and genetic factors. The prevalence of HL is higher in certain developing countries such as many African countries. In São Tomé and Príncipe, the deaf community is estimated to comprise less than 1% of the population based on the National Institute of Statistics of São Tomé and Príncipe (INE, 2014). Mutations in the *GJB2* gene (encoding connexin 26) are responsible for a significant proportion of nonsyndromic hearing loss (NSHL) cases in

several populations. Two large deletions, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), truncating the *GJB6* gene (encoding connexin 30), are also responsible for NSHL in some populations, being mostly found in *trans* with *GJB2* mutations (del Castillo et al., 2003, 2005). These genes map to 13q11-q12, and both of them are located within the *DFNB1* locus, with the first locus defined for nonsyndromic autosomal recessive HL. Loci for nonsyndromic autosomal recessive HL are designated by DFNB followed by a suffix integer (Smith and Van Camp, 1998). Mutations in *GJB2* reportedly do not play a significant role in the etiology of HL in Sub-

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Saharan African populations or their descendant populations (Bosch et al., 2014b; Javidnia et al., 2014; Lasisi et al., 2014; Shan et al., 2010). The role of del(*GJB6*-D13S1830) *GJB6* large deletion in NSHL in these populations is so far null (Bosch et al., 2014a; Kabahuma et al., 2011; Samanich et al., 2007; Shan et al., 2010). Regarding the del(*GJB6*-D13S1854) *GJB6* deletion, its presence has been investigated in Nigerian HL patients (Lasisi et al., 2014) and in HL patients of a predominantly Caribbean Hispanic and African descent (Shan et al., 2010), without positive results.

São Tomé and Príncipe, a former Portuguese colony, was formerly settled first by people from different regions of Sub-Saharan Africa, mostly slaves from the Gulf of Guinea, Congo, and Angola, brought to work in local plantations, and, to a minor extent, Portuguese who were involved in the slave trade between Africa and the Americas. In the first centuries after the discovery of São Tomé and Príncipe, besides the Portuguese, other Europeans were involved in the slave trade along the coast of Africa, namely the French, Spanish, Dutch, and English (Neves, 1989). In fact, São Tomé and Príncipe's population has been shown to present  $10.7\% \pm 0.9\%$  of European (mainly Portuguese) admixture (Tomás et al., 2002). Therefore, a putative role of *GJB2*, or even of the del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) large *GJB6* deletions, in the HL observed in São Tomé and Príncipe might potentially be anticipated.

The aim of the present study was to examine the genetic basis of HL in this understudied Sub-Saharan African island population, and so as to obtain a broader insight on the role of genetic contributions to this important disease impacting global health.

## Methods and Materials

### Subjects

A total of 316 individuals (136 HL patients and 180 controls), all of whom were born in São Tomé and Príncipe, ranging from 2 to 35 years old, participated in this study. The subjects were recruited during consultation provided by the humanitarian missions in São Tomé and Príncipe, at hospitals, schools, and a hotel, over a period from February 2012 to May 2014, constituting a convenience sample. All patients and controls answered a clinical questionnaire identifying risk factors (family history of HL, consanguinity, malaria infection, prenatal and perinatal history, and history of other infections), clinical history, and otolaryngology observation.

The patients presented with mild to profound nonsyndromic sensorineural hearing loss (NSSHL), which was bilateral HL in 92 individuals and unilateral HL in 44 individuals. All the control individuals had normal hearing in both ears. The patients and control samples did not display a significant difference in gender ( $p = 0.233$ ) and age ( $p = 0.271$ ).

The classification of HL was adopted from the World Health Organization (WHO, 2013). It considers the mean value of hearing threshold (considering the 0.5, 1, 2, and 4 kHz frequencies) in the better ear. The individual has HL when the best ear presents a hearing threshold higher than 25 dB and is graded after that with mild (26–40 dB), moderate (41–60 dB), severe (61–80 dB), and profound (81 dB or greater) (WHO, 2013).

The project was reviewed and approved by the Medical Ethics Committee of São Tomé and Príncipe and the Ethics Research Committee NMS|UNL (n°02/2014/CEFCM).

### Audiological examination

All 316 individuals were evaluated regarding their hearing status with a pure tone audiogram—Madsen Midimate 622 or auditory brainstem response—Vivosonic Integrity V500 audiometer depending on collaboration. The audiometric exams were carried out without an audiometric cabin, with earphones TDH39, in a closed room, with a level of noise measured by iPhone de SchabelDoesIT GbR, Munich, Germany (version 1.0.0), considered acceptable, based on ANSI S3.1-1999 (R2013). Electrophysiological thresholds were translated into the audiometric thresholds for frequencies 2 and 4 kHz, without applying any correction factor (Gorga et al., 1985, 2006; Jerger and Mauldin, 1978; van de Drift et al., 1987).

### DFNB1 molecular analysis

Peripheral blood samples were collected in Guthrie paper cards after informed consent had been signed. Approval of the Medical Ethics Committee was also obtained. Genomic DNA was extracted from each blood sample by using a commercially available kit (QIAamp® DNA micro kit; Qia-gen) according to the manufacturer's instructions. All DNA samples were stored at  $-20^{\circ}\text{C}$  until analysis.

Of the 316 subjects, 311 were successfully analyzed by sequencing regarding the *GJB2* coding region, and by multiplex polymerase chain reaction (PCR) (del Castillo et al., 2005) regarding the presence of the two *GJB6* large deletions, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854). These 311 individuals comprise 134 patients (90 bilateral and 44 unilateral) and 177 controls, matched by sex and age. PCR amplification and sequencing of the coding region of the *GJB2* gene was performed using previously described primers (Matos et al., 2010). The *GJB2* fragment that was amplified comprises the coding region and flanking noncoding regions, including the acceptor splice site. However, the extension of the sequence obtained beyond the coding region was variable, not allowing results from the acceptor splice site for all the subjects.

All electrophoretograms were visually inspected; the low-quality extremities were trimmed off; and heterozygosities were marked, using the Chromas Lite software (v.2.01). The resulting analyzed and edited sequences were copied from Chromas Lite in Fasta format and blasted against the reference sequence NG\_008358.1 using NCBI's Blast program (suite 2-sequences). All the variants described here were named according to the recommendations of the Human Genome Variation Society.

### Statistical analyses

A chi-square test was performed, and  $p$ -values were calculated using the SPSS v.20 software. When chi-square was not possible, we adopted Fisher's exact test. Hardy-Weinberg equilibrium test was performed using Court Lab's HW calculator. This test was only performed for c.\*84T>C, c.\*104A>T, and c.\*111C>T, since these were the only variants whose respective genotypes were all observed in five or more individuals.

## Results

Insofar as the *GJB2* sequencing is concerned, all the analyzed subjects were sequenced between c.-2 and c.\*6 positions, although most sequences extended, with quality, several nucleotides before c.-2 and after c.\*6. The sequencing results

TABLE 1. *GJB2* CODING VARIANTS IDENTIFIED IN PATIENTS AND CONTROLS (ALL IN HETEROZYGOSITY) AND RESPECTIVE CARRIER FREQUENCIES

<i>GJB2</i> coding variants	Bilateral HL	Unilateral HL	Controls
c.35delG (p.Gly12Valfs)	0/90	0/44	<b>1/177 (0.28%)</b>
c.101T>C (p.Met34Thr)	<b>1/90 (0.56%)</b>	0/44	0/177
c.109G>A (p.Val37Ile)	0/90	0/44	<b>1/177 (0.28%)</b>
c.186C>T (p.Asn62=)	0/90	0/44	<b>1/177 (0.28%)</b>
c.225G>T (p.Arg75=)	<b>1/90 (0.56%)</b>	0/44	<b>5/177 (1.41%)</b>
c.380G>A (p.Arg127His)	<b>1/90 (0.56%)</b>	0/44	<b>1/177 (0.28%)</b>
c.457G>A (p.Val153Ile)	0/90	0/44	<b>1/177 (0.28%)</b>
c.499G>A (p.Val167Met)	<b>1/90 (0.56%)</b>	<b>1/44 (1.14%)</b>	<b>1/177 (0.28%)</b>
Frequency of mutated alleles	4/180 (2.22%)	1/88 (1.13%)	11/354 (3.11%)

HL, hearing loss.

Boldface = carrier frequencies &gt; 0.

allowed for the identification of eight coding variants (Table 1) and 10 noncoding variants (Table 2) in patients and/or controls.

#### Coding variants of *GJB2*

Eight different coding variants, all in heterozygosity, have been identified in this study, in patients and/or control individuals (Table 1). None of these patients harbored any mutation in the acceptor splice site.

#### Noncoding variants of *GJB2*

We have identified 10 noncoding variants in the subjects of this study, being the genotyping results presented in Table 2. The most commonly identified variant in bilateral and unilateral HL patients and controls was c.\*84T>C, presenting in the latter group an allelic frequency of 51.36%. This variant as well as c.\*104A>T and c.\*111C>T are in Hardy–Weinberg equilibrium in the control group.

TABLE 2. *GJB2* NONCODING VARIANTS IDENTIFIED IN THE SUBJECTS AND RESPECTIVE GENOTYPIC FREQUENCIES

<i>GJB2</i> noncoding variants	Genotypes	Bilateral HL	Unilateral HL	Controls
c.-22-12C>T	CC	59.4% (19/32)	60% (6/10)	66.7% (26/39)
	CT	31.3% (10/32)	40% (4/10)	30.8% (12/39)
	TT	9.4% (3/32)	0% (0/10)	2.6% (1/39)
c.-15C>T	CC	82.7% (67/81)	85.7 (36/42)	88% (146/166)
	CT	16% (13/81)	14.3% (6/42)	11.4% (19/166)
	TT	1.2% (1/81)	0% (0/42)	0.6% (1/166)
c.-14G>A	GG	98.8% (80/81)	100% (43/43)	99.4% (165/166)
	GA	1.2% (1/81)	0% (0/43)	0.6% (1/166)
	AA	0% (0/81)	0% (0/43)	0% (0/166)
c.-7G>A	GG	100% (88/88)	100% (44/44)	99.4% (175/176)
	GA	0% (0/88)	0% (0/44)	0.6% (1/176)
	AA	0% (0/88)	0% (0/44)	0% (0/176)
c.-6T>A	TT	100% (89/89)	100% (44/44)	97.7% (172/176)
	TA	0% (0/89)	0% (0/44)	2.3% (4/176)
	AA	0% (0/89)	0% (0/44)	0% (0/176)
c.*78A>T	AA	97.6% (80/82)	100% (40/40)	100% (152/152)
	AT	2.4% (2/82)	0% (0/40)	0% (0/152)
	TT	0% (0/82)	0% (0/40)	0% (0/152)
c.*84T>C	TT	21.3% (17/80)	30% (12/40)	24.5% (36/147)
	TC	36.3% (29/80)	47.5% (19/40)	48.3% (71/147)
	CC	42.5% (34/80)	22.5% (9/40)	27.2% (40/147)
c.*96A>G	AA	100% (54/54)	100% (29/29)	99.2% (122/123)
	AG	0% (0/54)	0% (0/29)	0.81% (1/123)
	GG	0% (0/54)	0% (0/29)	0% (0/123)
c.*104A>T	AA	57.7% (30/52)	79.3% (23/29)	68.1% (79/116)
	AT	40.4% (21/52)	17.2% (5/29)	29.3% (34/116)
	TT	1.9% (1/52)	3.4% (1/29)	2.6% (3/116)
c.*111C>T	CC	57.1% (28/49)	79.3% (23/29)	67% (75/112)
	CT	42.9% (21/49)	17.2% (5/29)	30.4% (34/112)
	TT	0% (0/49)	3.4% (1/29)	2.7% (3/112)

TABLE 3. GENOTYPIC DISTRIBUTION REGARDING THE c.\*84T>C VARIANT IN CONTROLS AND PATIENTS WITH BILATERAL, SEVERE, OR PROFOUND HEARING LOSS (CHI-SQUARE TEST;  $P=0.005$ )

	c.*84T>C			Total
	TT	TC	CC	
Controls	36	71	40	147
Expected count	38.4	61.3	47.3	
Patients	16	12	24	52
Expected count	13.6	21.7	16.7	
Total	52	83	64	199

We have observed a statistically significant difference in the distribution of genotypes regarding c.\*84T>C between bilateral HL patients and controls when considering only the cases with severe and profound deafness (Table 3;  $p=0.005$ ). When also including the moderate bilateral HL patients, the difference in genotypic distribution did not remain statistically significant for the c.\*84T>C variant ( $p=0.101$ ).

#### GJB6 deletions

The del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) *GJB6* deletions have not been identified in the 134 patients (90 bilateral +44 unilateral) or 177 controls analyzed.

TABLE 4. ALL *GJB2* VARIANTS IDENTIFIED IN THIS STUDY AND THE POPULATIONS, PER THE 1000 GENOMES PROJECT (PHASE 3), IN WHICH THE ATTENDANT VARIANTS HAVE BEEN OBSERVED

GJB2 variant	dbSNP ID	Change at protein level/ Location	Effect	1000 genomes project (phase 3) populations <sup>a</sup>
c.-22-12C>T	rs9578260	Intron	Polymorphism <sup>b</sup>	ACB, ASW, CLM, ESN, ITU, LWK, MSL, MXL, PEL, PUR, YRI
c.-15C>T	rs72561725	5'UTR	Polymorphism <sup>b</sup>	ACB, ASW, CEU, CLM, ESN, GWD, LWK, MSL, MXL, PUR, YRI
c.-14G>A	rs367567291	5'UTR	Unknown	n.a.
c.-7G>A	rs398123813	5'UTR	Unknown	n.a.
c.-6T>A	rs148136545	5'UTR	Unknown	ASW, CLM, ESN, LWK, MSL, PUR
c.35delG	rs80338939	p.Gly12Valfs	Pathogenic <sup>c</sup>	BEB, CEU, CLM, FIN, GBR, IBS, MXL, TSI
c.101T>C	rs35887622	p.Met34Thr	Pathogenic <sup>d</sup>	ASW, CEU, CLM, FIN, GBR, IBS, MXL, PUR, TSI
c.109G>A	rs72474224	p.Val37Ile	Pathogenic <sup>c</sup>	CDX, CHB, CHS, CLM, JPT, KHV, LWK, MXL
c.186C>T	rs397516869	p.Asn62=	Unknown	n.a.
c.225G>T	rs149137695	p.Arg75=	Unknown	n.a.
c.380G>A	rs111033196	p.Arg127His	Controversial	BEB, GBR, GIH, ITU, KHV, PJL, STU, TSI
c.457G>A	rs111033186	p.Val153Ile	Controversial	BEB, CLM, GIH, ITU, PJL, STU, TSI
c.499G>A	rs111033360	p.Val167Met	Likely pathogenic <sup>c</sup>	LWK
c.*78A>T	rs576671031	3'UTR	Unknown	ACB
c.*84T>C	rs3751385	3'UTR	Polymorphism <sup>f</sup>	All populations
c.*96A>G	rs188027627	3'UTR	Unknown	ESN, LWK
c.*104A>T	rs7337074	3'UTR	Polymorphism <sup>b</sup>	ACB, ASW, CLM, ESN, GWD, IBS, LWK, MSL, MXL, PEL, PUR, YRI
c.*111C>T	rs7329857	3'UTR	Polymorphism <sup>b</sup>	ACB, ASW, CLM, ESN, GIH, GWD, IBS, LWK, MSL, MXL, PEL, PUR, YRI

Unknown—The authors consider that there are still insufficient data in the literature, including this study, for inferring benignity or pathogenicity of the variant.

Controversial—In view of the conflicting data in the literature regarding the pathogenicity of the variant, the authors are unable to classify it as either benign (polymorphism) or pathogenic.

<sup>a</sup>One thousand Genome Project's Populations: ACB, African Caribbeans in Barbados; ASW, Americans of African Ancestry in SW USA; BEB, Bengali from Bangladesh; CDX, Chinese Dai in Xishuangbanna, China; CEU, Utah Residents (CEPH) with Northern and Western European Ancestry; CHB, Han Chinese in Beijing, China; CHS, Southern Han Chinese; CLM, Colombians from Medellin, Colombia; ESN, Esan in Nigeria; FIN, Finnish in Finland; GBR, British in England and Scotland; GIH, Gujarati Indian from Houston, Texas; GWD, Gambian in Western Divisions in The Gambia; IBS, Iberian population in Spain; ITU, Indian Telugu from the UK; JPT, Japanese in Tokyo, Japan; KHV, Kinh in Ho Chi Minh City, Vietnam; LWK, Luhya in Webuye, Kenya; MSL, Mende in Sierra Leone; MXL, Mexican Ancestry from Los Angeles USA; PEL, Peruvians from Lima, Peru; PJL, Punjabi from Lahore, Pakistan; PUR, Puerto Ricans from Puerto Rico; STU, Sri Lankan Tamil from the UK; TSI, Toscani in Italy; YRI, Yoruba in Ibadan, Nigeria.

<sup>b</sup>Significant allelic frequencies in some 1000 Genome Project's populations and homozygous genotypes have been observed in normal-hearing controls.

<sup>c</sup>The pathogenicity of the variant is well established in the literature.

<sup>d</sup>In spite of OMIM considering this variant as one of unknown significance ([www.omim.org/entry/121011](http://www.omim.org/entry/121011)), we considered it as pathogenic based on several reports from the literature.

<sup>e</sup>Based on *in silico* analytic tools as assessed at the Deafness Variation Database (<http://deafnessvariationdatabase.org/>).

<sup>f</sup>This variant is clearly a polymorphism based on the genotypic frequencies available for several populations (dbSNP at NCBI).

n.a., not available.

## Discussion

HL remains an important global health burden. We highlight and contextualize the salient findings and conclusions from the present study.

### Coding variants of GJB2

**Pathogenic and controversial variants.** In this study, with regard to São Tomé and Príncipe's population, we have identified the pathogenic c.35delG, p.Met34Thr and p.Val37Ile mutations, and the controversial p.Arg127His and p.Val153Ile variants (Table 4). These five sequence changes are the most commonly found in patients from Eurasian and Mediterranean populations, and all have been previously found in Portuguese HL patients (Matos et al., 2013). Thus, a non-Sub-Saharan genetic influence in São Tomé and Príncipe's population is suggested, and the European genetic influx reported by Tomás et al. (2002), most likely due to admixture with the Portuguese, is supported by our results.

**p.Val167Met:** a likely recessive pathogenic variant. As regards the p.Val167Met variant, it has been previously found, in heterozygosity, in four Kenyans with prelingual, non-syndromic HL (Gasmelseed et al., 2004) and in one Cameroonian HL patient (Bosch et al., 2014b). This variant has also been observed in the Luhya (Webuye, Kenya) population (Table 4), from the 1000 genomes project ([www.1000genomes.org/](http://www.1000genomes.org/)), in heterozygosity and with an allelic frequency of 0.51%. The p.Val167Met variant, carried by one control individual from our sample, was also present in 1 out of 188 African-American control chromosomes (Samanich et al., 2007). The p.Val167Met variant has also been identified, in heterozygosity, in two studies, including African-American subjects, but the ethnicities of the carriers were not disclosed (Putcha et al., 2007; Ross et al., 2007). *In silico* analytic tools, as accessed at the Deafness Variation Database (<http://deafnessvariationdatabase.org/>), suggest the pathogenicity of p.Val167Met. Taking all together, p.Val167Met seems to be a recessive pathogenic variant of Sub-Saharan African origin.

**Synonymous variants.** We have identified two synonymous variants as well. The p.Arg75= variant was identified in one bilateral HL patient and in five controls (5/177 alleles = 1.41%), and the p.Asn62= variant was found in one control individual (1/354 alleles = 0.28%). Noteworthy, these two synonymous *GJB2* coding variants, of as yet unknown significance (Table 4), are present in Sub-Saharan African and Eastern Asian populations (Bosch et al., 2014b; Chen et al., 2014; Gasmelseed et al., 2004; Han et al., 2008; Mingkun et al., 2007; Trotta et al., 2011).

### Noncoding variants of GJB2

As expected, the São Tomé and Príncipe's population harbors some *GJB2* noncoding variants that are shared mainly with Sub-Saharan African populations and populations of a Sub-Saharan African ancestry. A statistically significant difference in genotypic distribution regarding the c.\*84T>C variant was observed between controls and patients with severe or profound bilateral HL ( $p=0.005$ ).

The most common of the 10 noncoding variants genotyped in the subjects of our sample, c.\*84T>C, is also found in the

project's populations of all the 1000 genomes (Table 4) and was observed in the control sample with an allelic frequency close to those of the Sub-Saharan populations as well as the Americans of African ancestry, Han Chinese and Japanese populations.

The c.\*84T>C variant (rs3751385) has been previously found to be significantly associated with HL (Dickson et al., 2010), in the context of demonstrating the creation of synthetic genome-wide associations by rare variants. In our sample, both allele T and C at the c.\*84 are common but a synthetic association between one or both c.\*84 alleles and HL cannot be excluded. A larger sample would be necessary to further investigate a putative association of variants at position c.\*84 with HL in São Tomé and Príncipe's population.

As regards the noncoding variants c.-22-12C>T and c.-15C>T, they were identified in controls with allelic frequencies of 17.95% and 6.33%, respectively. These polymorphisms are the most frequent in Sub-Saharan African populations and in populations of a Sub-Saharan African ancestry ([www.1000genomes.org/](http://www.1000genomes.org/); Table 4).

The c.-6T>A variant, observed in controls with an allelic frequency of 1.14%, is also the most frequent in Sub-Saharan African populations and in populations of a Sub-Saharan African ancestry ([www.1000genomes.org/](http://www.1000genomes.org/)). To the extent of our knowledge, this variant, of an unknown effect (Table 4), has only been described in the heterozygous state (Al-Qahtani et al., 2010; Shan et al., 2010; Tang et al., 2006; [www.1000genomes.org/](http://www.1000genomes.org/)).

Some other noncoding variants, of an unknown effect (Table 4), were rarely observed in this study. The c.-7G>A, c.-14G>A, c.\*78A>T, and c.\*96A>G variants were each observed in our sample only once or twice, in heterozygosity. The c.\*96A>G variant was observed only in the Luhya (Webuye, Kenya) and Essan (Nigeria) populations, in the heterozygous form. The c.\*78A>T variant has been observed only in the African Caribbean (Barbados) population (Table 4), also in the heterozygous form. No populational data are available for the c.-7G>A and c.-14G>A variants (Table 4).

### GJB6 deletions

None of the two large *GJB6* deletions has been identified in São Tomé and Príncipe. These results are similar to those obtained in previous studies on HL patients from Sub-Saharan African populations and Sub-Saharan African ancestry populations.

The del(*GJB6*-D13S1854) is not particularly frequent in Portuguese NSSHL patients (0.4% of the patients' alleles), and the del(*GJB6*-D13S1830) seems to be fairly rare in these patients (Chora et al., 2010; Matos et al., 2013). Given the relatively low degree of European (mainly Portuguese) admixture of São Tomé and Príncipe's population (Tomás et al., 2002), it is likely that none of the *GJB6* deletions plays a relevant role in NSSHL in São Tomé and Príncipe's population, as suggested by our data.

## Conclusions

The role of *GJB2* coding mutations in NSSHL in São Tomé and Príncipe seems to be of little significance. Our study, however, suggests the existence of pathogenic, and likely pathogenic, coding variants in São Tomé and Príncipe's population. Thus, although no biallelic HL patients have been

identified in our sample, the eventual occurrence of *GJB2*-related HL in this population should not be disregarded. The role of the *GJB6* large deletions in NSSHL in São Tomé and Príncipe, if any, is predicted by this study to be small.

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## Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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#### Abbreviations Used

ANSI = American National Standards Institute  
DNA = deoxyribonucleic acid  
GJB2 = gap junction protein beta 2  
GJB6 = gap junction protein beta 6  
HL = hearing loss  
NCBI = National Center for Biotechnology Information  
NMS|UNL = NOVA Medical School | Universidade Nova de Lisboa  
NSHL = nonsyndromic hearing loss  
NSSHL = nonsyndromic sensorineural hearing loss  
PCR = polymerase chain reaction  
WHO = World Health Organization