DFNB1 locus analysis in São Tomé and Príncipe population

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Introduction

Deafness is one of the leading causes of disability in the world. The prevalence of hearing loss is highest in South Asia and sub-Saharan Africa[1]. This condition could be caused by environmental conditions, genetics, aging, or a combination of these factors[2]. Congenital deafness occurs in approximately 1 in 1,000 live births; while 50% of these cases are hereditary in developed countries, the contribution of genetic mutations to deafness decreases in underdeveloped countries due to the higher relevance of environmental causes. *GJB2* (connexin 26) mutations have been identified as the most common cause of hereditary hearing loss in many populations[3]. Two large *GJB6* deletions, del(*GJB6*-D13S1830) and del (*GJB6*-D13S1854), are also a significant cause of HL in several countries. These two genes comprise the DFNB1 locus, which has sparsely been investigated in African HL patients.

In this study we aimed at estimating the prevalence of *GJB2* coding mutations and the two large *GJB6* deletions in a sample of São Tomé and Príncipe, It is the first study of this kind carried out in this population.

São Tomé and Príncipe is a small island in sub-Saharan Africa, near equator, discovered by Portuguese navigators in the early 1470s. It was a central warehouse for the assemblage and redistribution of slaves, first within the limits of the Gulf of Guinea, and later in the intercontinental trade with the Americas, especially with Brazil[4]. The peopling of São Tomé might have provided one of the first examples of the combination of diverse African contributions and European admixture that emerged from the overseas population relocations promoted by the Atlantic slave trade.

Material and Methods

Subjects

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

Audiological examination

- All 316 individuals were evaluated regarding their hearing status with Pure Tone Audiogram (PTA) - Madsen Midimate 622 or Auditory Brainstem Response (ABR) - 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).

DFNB1 analysis

- Peripheral blood samples were collected in *Guthrie* paper cards after informed consent being signed. Approval of the Medical Ethics Committee was previously obtained. Genomic DNA was obtained from each blood sample using a commercially available kit (QIAamp® DNA micro kit, Qiagen) according to the manufacturer's instructions. All DNA samples were stored at –20 °C until analysis. Of the 316 subjects, sequencing of the coding region of the *GJB2* gene was performed in 101 patients (66 bilateral + 35 unilateral) and 148 controls. These 249 subjects, as well as other 25 bilateral HL patients, 9 unilateral HL patients and 31 controls, were tested for both *GJB6* deletions.

- The *GJB2* coding region was amplified in a single fragment of 928 bp, as previously described[5]. The PCR products were purified with the JetQuick PCR Purification Spin Columns kit, and subsequently sequenced by an outsource company. The presence of the *GJB6* deletions was investigated by multiplex PCR, as described by del Castillo *et al* 2005[6].

Results and Discussion

Sequencing results allowed for the identification of coding variants (table 1) and also for the determination of genotypic frequencies of ten noncoding variants (table 2).

Some pathogenic and likely pathogenic *GJB2* coding mutations have been identified in this study:

Bilateral HL (2/132 alleles)
- p.Met34Thr - pathogenic variant
- p.Lys112Met - likely pathogenic variant (based in in silico prediction at http://deafnessvariationdatabase.org/)

Unilateral HL (1/70 alleles)
- p.Val167Met - likely pathogenic variant (based in silico prediction at the same website)

Controls (2/296 alleles)
- p.Val37Ile - pathogenic variant
- p.Val167Met - likely pathogenic variant

Two synonymous changes p.Arg75= and p.Asn62= have been identified in HL patients and/or controls.

The non-sub-Saharan genetic influence in São Tomé and Príncipe population, most likely due to admixture with Portuguese, was attested by the identification of coding mutations commonly found in Europe and Asia: p.Met34Thr, p.Val37Ile, p.Arg127His and p.Val153Ile. Interestingly, the three groups of subjects and the genotype concerning the c.*84T>C variant are not independent, as shown in table 2 (Chi-square test, p=0.036). Indeed, the allele c.*84C is overrepresented in the bilateral HL patients compared with the controls (Chi-square test, p=0.011).

Our study suggests that the contribution of *GJB2* coding mutations to HL in São Tomé and Príncipe is small, similarly to what has been observed in other studies of sub-Saharan populations[2]. This is so despite the admixture to some degree with European populations, namely the Portuguese[4].

None of the *GJB6* deletions was identified in the 91 bilateral HL patients, 44 unilateral HL patients or 179 controls analyzed.

Table 1. *GJB2* coding variants identified in patients and controls (all in simple heterozygosity), and respective carrier frequencies.

GJB2 variants	Bilateral HL	Unilateral HL	Controls
c.101T>C (p.Met34Thr)	**1/66**	0/35	0/148
c.109G>A (p.Val37Ile)	0/66	0/35	**1/148**
c.186C>T (p.Asn62=)	0/66	0/35	**1/148**
c.225G>T (p.Arg75=)	**1/66**	0/35	**4/148**
c.335A>T (p.Lys112Met)	**1/66**	0/35	0/148
c.380G>A (p.Arg127His)	0/66	0/35	**1/148**
c.457G>A (p.Val153Ile)	0/66	0/35	**1/148**
c.499G>A (p.Val167Met)	0/66	**1/35**	**1/148**

Table 2. *GJB2* noncoding variants identified in patients and controls, and respective genotypic frequencies.

GJB2 noncoding variant	Genotypes	Bilateral HL	Unilateral HL	Controls	*P-Value (Chi-Square test)*
c.-22-12C>T	CC	13/22	5/9	14/21	0.651*
	CT	6/22	4/9	6/21	
	TT	3/22	0/9	1/21	
c.-15C>T	CC	51/61	28/34	124/142	0.487*
	CT	9/61	6/34	18/142	
	TT	1/61	0/34	0/142	
c.-14G>A	GG	0/61	0/34	0/143	NA
	GA	0/61	0/34	1/143	
	AA	0/61	0/34	0/143	
c.-7G>A	GG	64/64	35/35	147/148	NA
	GA	0/64	0/35	1/148	
	AA	0/64	0/35	0/148	
c.-6T>A	TT	66/66	35/35	144/148	0.277*
	TA	0/66	0/35	4/148	
	AA	0/66	0/35	0/148	
c.*78A>T	AA	56/58	32/32	126/126	NA
	AT	2/58	0/32	0/126	
	TT	0/58	0/32	0/126	
c.*84T>C	TT	10/57	9/32	34/122	**0.036**
	TC	19/57	15/32	56/122	
	CC	28/57	8/32	32/122	
c.*96A>G	AA	0/35	0/21	0/96	NA
	AG	0/35	0/21	1/96	
	GG	0/35	0/21	0/96	
c.*104A>T	AA	18/33	17/21	66/93	0.213*
	AT	14/33	3/21	24/93	
	TT	1/33	1/21	3/93	
c.*111C>T	CC	17/31	17/21	62/89	0.129*
	CT	14/31	3/21	24/89	
	TT	0/31	1/21	3/89	

NA – Not Applicable; *Chi-Square Test by Monte Carlo Simulation

Conclusions

- The role of *GJB2* coding mutations in HL in São Tomé and Príncipe seems to be of little significance.
- Our study, however, shows the existence of pathogenic and likely pathogenic coding variants in São Tomé and Príncipe population.
- Thus, although no bi-allelic HL patients have been identified in our sample, the occurrence of *GJB2*-related HL in this population is possible. The prevalence of either the two large *GJB6* deletions, if present, is expected to be less than 1/91 (1,1%) in bilateral HL patients from São Tomé and Príncipe.

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