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Non-syndromic hearing impairment in a multi-ethnic population of Northeastern Brazil

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ABSTRACT

Objective: There are many hearing impaired individuals in Monte Santo, a rural municipality in the state of Bahia, Brazil, including multiple familial cases strongly suggestive of a genetic aetiology.
Methods: The present study investigated 81 subjects with hearing impairment (HI) recruited from 36 families. Mutations often associated with HI, i.e. the DFNB1 mutations c.35delG in *GJB2*, deletions del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), and A1555G in the mitochondrial gene *MTRNR1* were initially analyzed, with additional mutations in *GJB2* identified by sequencing the coding region of the gene.
Results: Seven different mutations were present in *GJB2* with mutations c.35delG and p.Arg75Gln, which are known to be pathogenic, identified in 37.0% of the subjects. Individuals homozygous for the c.35delG mutation were diagnosed in eight families, corresponding to 24.7% of unrelated individuals with nonsyndromic hearing impairment (NSHI), and an additional heterozygote for this mutation was present in a single family. Ten individuals (12.4%) in another family were heterozygous for the mutation p.Arg75Gln.
Conclusions: Significant heterogeneity was observed in the alleles and patterns of NSHI inheritance among the subjects studied, probably due to the extensive inter-ethnic admixture that characterizes the peoples of Brazil, together with a high prevalence of community endogamy and consanguineous marriage.

1. Introduction

Hearing impairment (HI) can result from genetic and/or environmental factors. In developed countries approximately 60% of cases are hereditary, while 30% are acquired and 10% have an undefined aetiology [1,2]. Of the hereditary forms of HI, nonsyndromic and syndromic disorders account for an estimated 70% and 30% of cases respectively [3,4]. Although no nationally representative data are available on the prevalence or aetiology of HI in Brazil, most causes appear to be environmental in nature [5,6]. Several studies investigating the genetic basis of HI have, however, demonstrated the presence of a range of mostly recessive mutations [7–11].

Hereditary HI is highly heterogeneous, and to date mutations associated with nonsyndromic hearing impairment (NSHI) have been described in more than 70 genes exhibiting different modes of inheritance [12,13]. Despite the large numbers of genes and mutations implicated the DFNB1 locus appears to be principally involved [14–17], with the genes *GJB2* and *GJB6* present at this locus encoding the proteins connexin 26 (Cx26) and connexin 30 (Cx30) [18–20]. More than 100 mutations associated with NSHI have been described in *GJB2*, with both autosomal recessive and autosomal dominant modes of inheritance [17]. The most common AR mutation associated with NSHI in Caucasian populations is c.35delG(p.Gly12fx) [21].

A high frequency of c.35delG mutation carriers has been reported in many European countries [21]. In São Paulo, Brazil this mutation was detected in 2.2% of 223 newborns studied [22], while

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another screening programme on 620 newborns also conducted in São Paulo indicated a carrier frequency for the mutation of 1.0% [23]. No previous molecular studies in individuals with HI have been conducted in northeastern Brazil, although the c.35delG mutation was identified in individuals of African ancestry with normal hearing ability in Salvador, the state capital of Bahia, where the overall carrier frequency of the mutation was estimated to be 1.0% [24].

The 309 kb deletion in *GJB6*, del(*GJB6*-D13S1830), was the second most frequent connexin mutation reported for NSHI in Spain, France, Israel, Great Britain and Brazil [20,25]. Individuals who are either homozygous for del(*GJB6*-D13S1830) or compound heterozygotes of del(*GJB6*-D13S1830) and a mutant allele of *GJB2* develop NSHI, resulting in profound to severe HI [20]. The 232 kb deletion del(*GJB6*-D13S1854) also leads to partial loss of *GJB6* and has frequently been described in individuals with NSHI in Italy, England and Brazil [26]. A number of studies have indicated that the pathogenic effect of the 309 kb and 232 kb deletions is due to the deletion of a cis-acting enhancer of *GJB2* expression [27–29]. Mutations in mitochondrial DNA have been associated with both syndromic and nonsyndromic HI [13,30]. The A1555G mutation in mitochondrial gene *MTRNR1* has been identified in hereditary NSHI and in aminoglycoside-induced HI in families from several countries [31–35]. The A1555G mutation also has been reported in cases of NSHI in Brazil [8,9,36].

Due to widespread inter-ethnic admixture between people of European, African and Amerindian ancestry in the 500 years since European colonization, and more recent admixture with Middle Eastern and East Asian migrants, the Brazilian population has been described as one of the most heterogeneous in the world [37]. With a total population approaching 200 million, ethnicity in different regions of Brazil reflects both historical patterns of settlement and internal migration related to economic dynamics. Within Bahia 76.3% of residents, mainly living in the coastal regions, self-identified as being of African ancestry in the 2010 Census [38]. By comparison, an increasing proportion of European ancestry is seen in communities resident in inland regions of the state [39].

The aim of the present study was to investigate the genetic aetiology of HI by analyzing mutations in genes *GJB2*, *GJB6* and *MTRNR1* in individuals with hearing impairment from Monte Santo, a multi-ethnic inland community in Bahia.

2. Subjects and methods

2.1. Subjects

According to the 2010 Census of Brazil, the predominantly rural municipality of Monte Santo had a population of 52,338 inhabitants resident in some 200 villages [38]. Eighty-one patients with HI recruited from 36 families resident in Monte Santo were investigated between 2008 and 2010. The study initially included individuals with both sporadic and familial histories of HI, and with prelingual and postlingual HI. However, individuals who presented with evidence of syndromic or postlingual HI but with no familial recurrence subsequently were excluded.

Audiological evaluations using a tympanometry test, estimations of acoustic reflex thresholds (0.5, 1, 2 and 4 kHz), pure tone audiometry thresholds and evoked auditory brainstem response (ABR) were performed in the School of Audiology Clinic of the Metropolitan Union of Education and Culture College (UNIME), located in Lauro de Freitas, Bahia, Brazil. Institutional Review Board (IRB) approval was obtained from Research Ethics Committee of the Gonçalo Moniz Research Center (CPqGM), Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Bahia, Brazil (Case No. 182/2008, Protocol No. 274). Informed written consent was obtained from all individuals with HI, their legal representatives, or adult members of their immediate families.

2.2. Molecular analysis

Molecular analyses were performed at the Laboratory for Advanced Public Health at CPqGM/FIOCRUZ in Salvador, Bahia, Brazil. Genomic DNA was extracted from peripheral blood leucocytes [40,41], with mutations in *GJB2*, the deletions del(*GJB6*-13S1830) and del(*GJB6*-D13S1854) in *GJB6*, and the mitochondrial mutation A1555G in *MTRNR1* investigated. All c.35delG/*GJB2* mutations were first analyzed by PCR-RFLP using the B*st*NI enzyme [42,43]. The genotypes of all individuals with c.35delG mutations were confirmed by sequencing, and the mutations del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) were investigated by multiplex assays [26]. The A1555G/*MTRNR1* mutation was investigated using a protocol described by Kupka et al. (2002) [44], and analyses of the other mutations, including c.35delG were performed by direct sequencing of the *GJB2* coding region [45].

3. Results

3.1. Family backgrounds of the study population

Preparatory genealogical investigations identified a total of 1479 individuals belonging to 36 large extended families, 31 of which reported recurrence of HI. Pedigree analysis indicated that 163 subjects with HI in the 36 extended families were of probable genetic origin, with 152 of these affected individuals resident in Monte Santo. Of the 81 people with HI recruited into the present study, 95.1% reported a family history of hearing defect. No specific information on consanguinity was available for 11 subjects, but 36/70 (52.2%) of the remaining cases were born to parents related as second cousins or closer ($F \geq 0.0156$) (Table 1). The average age of participants in the study was 32 years (range: 2–70 years), 56.0% were male, 33.3% self-identified as White and 66.7% as *Pardo* (mixed ethnic ancestry).

3.2. Audiological assessment

In each of the study subjects the pattern of HI observed by imittance audiometry was suggestive of sensorineural HI, with all individuals presenting with bilateral HI and 94.0% reporting prelingual HI. The average age at initial diagnosis of HI in the prelingual cases was 2.5 years (range: 1 month to 12 years). In the five reported cases of postlingual HI the average age of HI

Table 1
Summary of genotypes identified by analysis of the coding region of *GJB2* and parental consanguinity in 81 individuals with HL from Monte Santo, Bahia, Brazil.

Genotype(s)	*N* (%)	Consanguinity		
		Yes (*N*)	No (*N*)	Unclear (*N*)
+/+	38 (46.9)	19	17	2
c.35delG/c.35delG[a]	20 (24.7)	11	7	2
p.Arg75Gln/+[a]	10 (12.4)	0	7	3
c.-22-12C>T/+	4 (4.9)	3	1	0
c.-22-12C>T/p.V27I	1 (1.2)	1	0	0
c.-15 C>T/+	1 (1.2)	1	0	0
c.35delG/+	1 (1.2)	0	0	1
p.Val27Ilep/+	4 (4.9)	0	2	2
p.Lys168Arg/+	1 (1.2)	0	0	1
c.*1C>T/+	1 (1.2)	1	0	0
Total	81 (100.0)	36	34	11

+ wild-type allele.
[a] Genotypes known to be pathogenic.