

## Original Article

# Strategies for genetic study of hearing loss in the Brazilian northeastern region

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**Abstract:** The overall aim of this study was to estimate the contribution of genetic factors to the etiology of hearing loss (HL) in two counties in the Brazilian northeastern region. A cross-sectional study, based on the key informant approach (KI) was conducted in Queimadas and Gado Bravo counties (Paraíba, Northeast Brazil). The sample consisted of 182 patients with HL. Genetic screening of the most frequent mutations associated with HL was performed for all samples. DFNB1 mutations were the most frequently found in both counties. The c.35delG mutation was detected in homozygosis in seven non-syndromic probands in Queimadas (7/76, 9.2%) and only a single homozygote with this mutation was found in Gado Bravo (1/44, 2.3%). We also detected the del(*GJB6*-D13S1854) mutation in non-syndromic probands from Gado Bravo (2/44, 4.5%). The c.189C>A (p.TyrY63\*) mutation in the *CLRN1* gene was detected in homozygosis in 21/23 Usher syndrome patients from Gado Bravo and it was not found in Queimadas. Cases with probable genetic etiology contributed approximately to half of HL probands in each county (54.6% in Gado Bravo and 45.7% in Queimadas). We confirm the importance of DFNB1 locus to non-syndromic HL but we show that the frequency of mutations in the northeastern region differs somewhat from those reported in southeastern Brazil and other populations. In addition, the extremely high frequency of individuals with Usher syndrome with c.189C>A variation in *CLRN1* indicates the need for a specific screening of this mutation.

**Keywords:** Epidemiology, hearing loss, DFNB1, *GJB2*, Usher syndrome, *CLRN1*

## Introduction

Hearing loss (HL) affects approximately 360 million people worldwide [1] with an incidence of 1 in 1,000 births [2]. In Brazil, about 2 million people are affected by this condition and approximately 650,000 of these live in the northeastern region [3]. The northeastern region is the poorest one in the country. The highest frequencies of consanguineous marriages are observed in this region [4, 5].

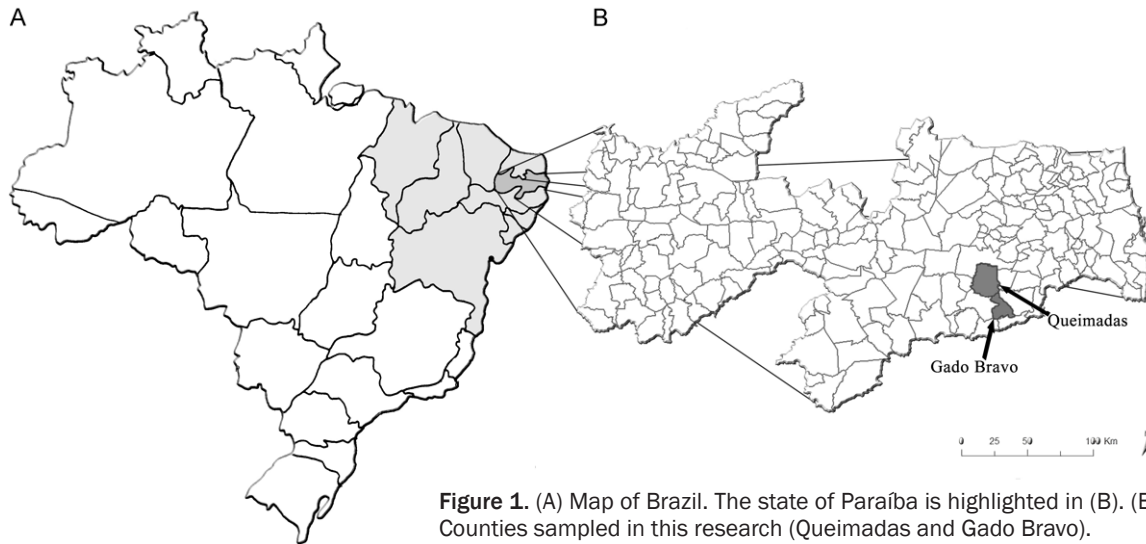
About half of all HL cases in developed countries are due to genetic factors, and 30% of these are syndromic cases [6]. More than 400 syndromes were described presenting HL associated with other clinical abnormalities [7], and Usher syndrome (US) is the most common type of autosomal recessive syndromic hearing loss (ARSHL) disorder [8, 9]. The prevalence of this

syndrome was estimated as 1 per 6,000 individuals in the United States [10]. Usher syndrome, characterized by combined retinitis pigmentosa with HL, can be classified in three main subtypes (USH1, USH2 and USH3), to which nine, four and two loci have been mapped, respectively, with a total of 11 genes having been identified up to now [11].

Among non-syndromic genetic cases, 80% have an autosomal recessive mechanism (ARNSHL) [12, 13]. The autosomal dominant mechanism corresponds to 14 to 24% of genetic cases; 2 to 3% are due to X-linked HL and 1% is due to maternal inheritance [9, 14].

The locus DFNB1, that houses *GJB2* and *GJB6* genes [15], is the most frequently associated with ARNSHL [16], the *GJB2* gene being responsible for about 50% of the recessive deafness

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**Figure 1.** (A) Map of Brazil. The state of Paraíba is highlighted in (B). (B) Counties sampled in this research (Queimadas and Gado Bravo).

cases [17]. In different populations, the most frequent mutation associated with HL is the c.35delG in the *GJB2* gene, found in homozygosis from 10% to 63% of non-syndromic HL cases [17, 18]. Other frequent mutations in this gene are the c.167delT, c.235delC, p.Trp24\* (or c.71G>A) and p.Arg143Trp (or c.427T>C), which are frequently found in Ashkenazi Jews, in Asian, Indian and African populations, respectively [16, 19-21].

Four large deletions near the *GJB6* gene were identified and they generally act as a second recessive mutation in the *DFNB1* locus in affected individuals [22-26]. The del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) are more frequently found in European and European-derived populations [23]. The second most important locus related to autosomal recessive HL is the *DFNB4* locus, which encodes the *SLC26A4* gene; more than 200 mutations in the coding region of this gene have been described [27, 28]. In the mitochondrial DNA, the most frequent mutation found among people with non-syndromic HL is m.1555A>G, in the *MTRNR1* gene [29].

In Brazil, the frequency of mutations in the *DFNB1* locus varies from 1% to 18.2% in non-syndromic hearing loss cases and the observed frequencies of c.35delG mutation in homozygosis ranged from 1.3% to 15.2% [30-38]. Most studies on genetic HL in Brazilian samples were concentrated in the southeastern region, and only one previous study was conducted in the northeastern region [37].

The overall aim of this study was to estimate the contribution of genetic factors to the etiology of HL in two counties located in the Brazilian northeastern region. The accurate characterization of causes of HL and the role of genetic factors may contribute to the establishment of public medical policies for these populations.

### Materials and methods

#### Population sample

A cross-sectional study, based on the key informant approach (KI), in order to detect disabilities in resource-poor countries [39-41], was conducted in the Queimadas and Gado Bravo counties, with 41,054 and 8,376 inhabitants, respectively [3], in the state of Paraíba, Northeast of Brazil (**Figure 1**). All individuals who had been detected by the Community Health Agents as presenting HL were invited to participate in genetic and audiological evaluation. Individuals presenting the first symptoms after 60 years of age were excluded.

The research was approved by the Ethics committees from Universidade Estadual da Paraíba (Process 0357.0.133.133-11) and from the Instituto de Biociências da Universidade de São Paulo (Process 126/2011 - FR. 458729).

The sample consisted of 182 patients with HL (106 patients from Queimadas and 76 from Gado Bravo), from which 136 were unrelated probands (81 from Queimadas and 55 from Gado Bravo). The Brazilian northeastern population is trihybrid, resulting from the admixture

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of European, African and Native American individuals. Most, if not all subjects from our sample, fall into this category. A semi-structured interview was conducted to collect clinical information and family data.

Audiological evaluation was carried out by audiologists from Universidade Federal da Paraíba. Fundoscopic examination was performed by an ophthalmologist from the Universidade Federal de Campina Grande in 33 patients from Gado Bravo who reported visual impairment associated with HL, in order to evaluate the presence of retinitis pigmentosa.

### *Molecular analysis*

DNA from blood samples of about 10 ml was extracted using the Autopure LS device by Genra Systems, available at the Centro de Estudos do Genoma Humano (USP, São Paulo, Brazil).

The c.35delG and c.167delT mutations in the *GJB2* gene, and m.1555A>G mutation in the mitochondrial gene (*MTRNR1*) were screened by polymerase chain reaction (PCR), followed by DNA digestion using restriction enzymes *Bst* I, *Pst* I and *Hae* III, respectively [16, 42, 43]. Digestion of the PCR products was performed with 10 units of each enzyme and the products were subjected to gel electrophoresis in a 6% polyacrylamide gel and visualized after staining with silver nitrate impregnation [44]. The del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) were detected by specific multiplex PCR [26] and the products were visualized on agarose gel after staining with 2% SYBR Safe (Life Technologies, Carlsbad, CA, USA). This screening was performed in all HL patients.

*GJB2* sequencing was performed to confirm the mutations detected in the previous screenings, either in homozygosis or heterozygosis. It was also performed to identify a second recessive mutation in samples from heterozygote individuals and in samples from probands of pedigrees with probable autosomal recessive or autosomal dominant inheritance. Two pairs of primers were used for *GJB2* gene sequencing (1F: 5'-3' ACC TGT GGT TTT GAG GTT GTG T and 1R: 5'-3' ACC TTC TGG GTT TTG ATC TCC TC; 2F: 5'-3' GGA AGT TCA TCA AGA AGG GGG and 2R: 5'-3' TGA GCA CGG GTT GCC CTC ATC). The reaction products were analyzed in the ABI

3730 DNA Analyzer equipment (Applied Biosystems, Carlsbad, CA, USA) at the Centro de Estudos do Genoma Humano, Departamento de Genética e Biologia Evolutiva (São Paulo, Brazil). The results were analyzed using the software Chromas Lite (<http://www.chromas-lite.softpedia.com>) and MEGA5 (<http://www.megasoftware.net>).

Genomic scanning was performed by 50K SNP arrays genotyping in samples of four siblings affected by Usher syndrome (GeneChip® Human Mapping Array Hind 240, Affymetrix, Santa Clara, CA, USA). The DNA was diluted to the original concentration of 50 ng/μl and 5 μl, and fragmented by *Hind*III, as per the manufacturer's protocol (Uniscience website; GeneChip Mapping 10K 2.0 Assay Manual). The analysis was performed with GeneChip Scanner 3000 equipment and the results were analyzed by GeneChip Operating Software (Affymetrix, Santa Clara, CA, USA).

The results of 50K SNP arrays were analyzed for obtaining multipoint LOD scores. The Alohomora software was used to convert the obtained data from the Affymetrix genotype platform into files for linkage analysis (Santa Clara, CA, USA), and Pedcheck was used for checking Mendelian segregation [45]. Merlin software was used to obtain multipoint LOD scores [46]. The disease was analyzed as an autosomal recessive trait and the disease allele frequency was 0.0001. Marker allele frequencies were calculated using the data from the family. The recombination rate was assumed to be similar in both genders. The mutation rate of the gene was considered to be zero.

Four microsatellites mapped near the *SLC26A4* gene were genotyped (D7S2420, D7S496, D7S2456 and D7S799) and also the molecular marker D7S2459, located at the intron 11 of this gene, was analyzed. This analysis was performed in samples from individuals from autosomal recessive hearing loss (ARHL) pedigrees without detected mutations in previous molecular screenings (49 samples from 24 pedigrees). The primers for amplification are described in databases (<http://genome.ucsc.edu/> and <http://www.ncbi.nlm.nih.gov/>), except for the D7S799 marker, for which the reverse primer was designed using PRIMER 3 (<http://primer3.sourceforge.net/>).

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**Table 1.** Demographic, socioeconomic data and individuals sampled in both counties

	Counties	
	Gado Bravo	Queimadas
Population (inhabitants)	8,376	41,049
Population Density (pop. per km <sup>2</sup> )	43.53	102.17
Human Development Index (HDI)	0.527	0.613
Gross Domestic Product (GDP) per capita in Dollars	US\$ 1,702	US\$ 2,119
Total of HL individuals from county (reported by CHA)	85 (1.01%)	150 (0.37%)
HL individuals sampled	76 (89.4%)	106 (70.7%)
HL individuals sampled in the total population of county	0.91%	0.26%

Microsatellites mapped near *MYO7A* (11q13) and *CLRN1* (3q25) genes were genotyped in four sibling samples affected by Usher syndrome: 11 microsatellites mapped near *CLRN1* gene (D3S1566, D3S3681, D3S1271, D3S1278, D3S1267, D3S1292, D3S1569, D3S1279, D3S1614, D3S1262 and D3S1580), and 11 microsatellites near *MYO7A* gene (D11S904, D11S935, D11S905, D11S4191, D11S987, D11S1314, D11S937, D11S4175, D11S898, D11S908 and D11S925). The primers are available from ABI PRISM® Linkage Mapping Set v. MD10-2.5 kit from Applied Biosystems (Foster City, CA, USA). The polymorphic fragments were subjected to capillary electrophoresis using the ABI 3730 DNA Analyzer apparatus (Applied Biosystems, Carlsbad, CA, USA) and analyzed using the GeneMapper software (Applied Biosystems, Carlsbad, CA, USA).

*SLC26A4* exon sequencing was performed in samples from three probands from pedigrees in which microsatellite segregation was compatible with *SLC26A4* linkage. The primers for amplification of the coding exons of this gene are described [47-49], with the exception of primers for amplification of exons 11, 12, 15 and 17, which were designed using PRIMER 3 (<http://primer3.sourceforge.net/>). All samples from Usher syndrome patients were subjected to *CLRN1* gene sequencing. Primers to amplify exon 1 were designed by PRIMER 3 (1F: 5'-3' GGC CGG AGT GTT CAG TTT T and 1R: 5'-3' TTT CAT ATG GTT CAC ACC GAT T). Primers for exons 2, 3, 3b and 3c were previously described [50, 51].

The MLPA technique was applied to samples of 10 probands with a single recessive mutation in heterozygosity in the *DFNB1* locus and in 2

3730 DNA Analyzer instrument (Applied Biosystems, Carlsbad, CA, USA). The results were analyzed using the Gene Marker software (<http://www.softgenetics.com/GeneMarker.html>).

### Results and discussion

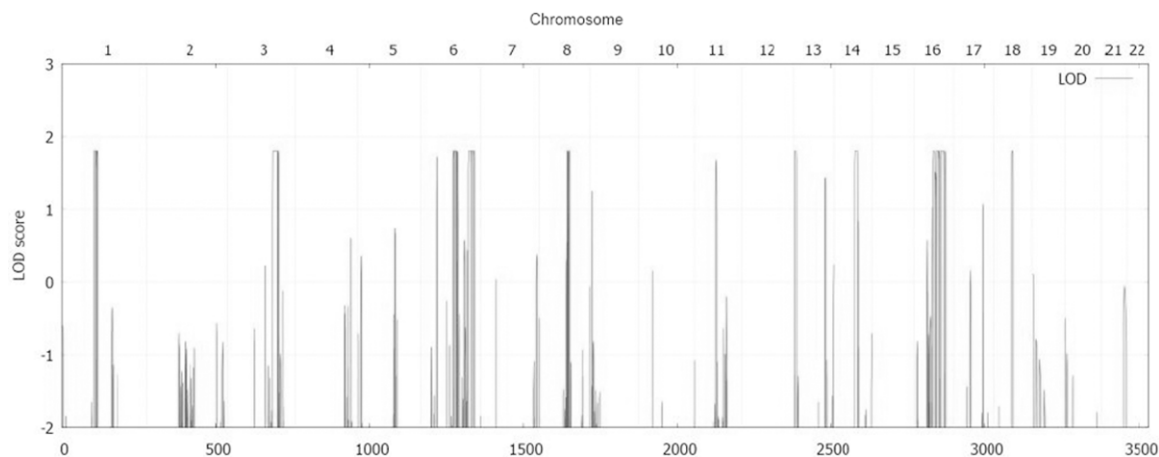
#### *Characterization of the populations*

**Table 1** lists demographic and socioeconomic data, and number of sampled individuals with HL in each county [3, 52]. The Brazilian census [3] indicated that there are about 650,000 people with hearing impairment in the northeastern region of Brazil, 48,000 of them in the state of Paraíba. In Gado Bravo and Queimadas counties, the census registered 232 (2.8%) and 427 (1.1%) people with HL respectively; these frequencies are higher than the ones reported in the present study (76 and 106; 0.91% and 0.27%, respectively). These discrepancies could be explained by the strategy of Brazilian census: information was registered without clinical confirmation; besides, people showing the first HL symptoms after 60 years were probably included.

Interestingly, the proportion of affected individuals in Gado Bravo was three times higher than Queimadas (0.91% and 0.27%, respectively). Excluding the Usher syndrome cases from both counties (23 patients from Gado Bravo and one patient from Queimadas), the frequency of HL cases in Gado Bravo is still two times higher than Queimadas (0.63% and 0.25%, respectively). The parental consanguinity rate did not explain the greater number of HL cases in Gado Bravo (21.8%) as compared to Queimadas (28.4%). Data for HDI and GDP per capita income of the two counties were compared [52]



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**Figure 2.** Multipoint parametric LOD score analyses from samples of four siblings with Usher syndrome. The map distance (cM) was converted (bp) using as reference the genome GRCh37/hg19.

**Table 2.** Mutation frequencies in the *GJB2*, *GJB6* and *MTRNR1* genes in the group of non-syndromic HL probands

Genes	Mutations	Counties			
		Gado Bravo		Queimadas	
		Homozygosis	Heterozygosis	Homozygosis	Heterozygosis
<i>GJB2</i>	c.35delG	1/44 (2.3%)	3/44 (6.8%)	7/76 (9.2%)	7/76 (9.2%)
	c.167delT	0/44	0/44	0/76	0/76
	p.Trp24* (c.71G>A)	0/44	3/44 (6.8%)	0/76	0/76
<i>GJB6</i>	del( <i>GJB6</i> -D13S1830)	0/44	0/44	0/76	0/76
	del( <i>GJB6</i> -D13S1854)	0/44	2/44 (4.5%)	0/76	0/76
<i>MTRNR1</i>	m.1555A>G	0/44	0/44	0/76	0/76

and both counties are grouped in the range of average HDI (500-799) and have similar GDP per capita income. They do not explain the differences in prevalence between both communities.

### Syndromic hearing loss

Syndromic HL was identified in 17 out of 136 probands (12.5%), being 12 from Gado Bravo and five from Queimadas. One patient of the former county had profound bilateral HL, telecanthus, synophrys, hypochromic bright blue color of the iris, and a frontal white hair forelock, being a case of Waardenburg type I syndrome (1/12, 8.3%). The remaining 11 probands from Gado Bravo had HL associated with retinitis pigmentosa (Usher syndrome). In Queimadas, three probands had mental retardation (3/5, 60%), one was affected by a polyneuropathy syndrome (1/5, 20%) and one proband had retinitis pigmentosa (US; 1/5, 20%).

The frequency of Usher syndrome in Gado Bravo was calculated as one per 364 individuals, about 16 times higher than in the US population prevalence [10]. Samples of four siblings affected by Usher syndrome from Gado Bravo were subjected to 50k SNP array genotyping assay and the corresponding result of the LOD score analysis is shown in **Figure 2**. Eleven regions with LOD scores near 1.8 were found (in chromosomes 1, 3, 6, 8, 11, 13, 14, 16 and 18). Two of these mapped regions had genes previously associated with Usher syndrome (*CLRN1* and *MYO7A* genes). The samples were subjected also to microsatellite genotyping with markers mapped near *CLRN1* and *MYO7A* genes. This allowed exclusion of *MYO7A* gene (11q13.5) as a candidate. Microsatellites near the region of *CLRN1* segregated with the phenotype and this gene was selected for sequencing. The c.189C>A (p.Tyr63\*) mutation, localized in exon 1 of this gene, was detected in homozygosis in all affected siblings. Samples

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**Table 3.** Genotype Frequencies in non-syndromic probands with HL

Genotypes	Number of non-syndromic probands with the genotype in the total sample		Number of non-syndromic probands with the genotype among the total of genotypes with pathogenic mutations	
	Queimadas	Gado Bravo	Queimadas	Gado Bravo
c.35delG/c.35delG	7/76 (9.2%)	1/44 (2.3%)	7/14 (50%)	1/7 (14.3%)
c.35delG/+	7/76 (9.2%)	1/44 (2.3%)	7/14 (50%)	1/7 (14.3%)
c.35delG/del(GJB6-D13S1854)	0/76	1/44 (2.3%)	0/14	1/7 (14.3%)
c.35delG/p.Trp24* (c.71G>A)	0/76	2/44 (4.5%)	0/14	2/7 (28.6%)
del(GJB6-D13S1854)/+	0/76	1/44 (2.3%)	0/14	1/7 (14.3%)
p.Trp24* (c.71G>A)/+	0/76	1/44 (2.3%)	0/14	1/7 (14.3%)

The signal + means wild sequence allele.

from the remaining Usher syndrome patients (19 from Gado Bravo and one from Queimadas) were also subjected to sequencing and 21/23 patients from Gado Bravo presented this mutation in homozygosity, which was not detected in the only affected subject from Queimadas. This mutation has been previously described in studies of Spanish populations [50, 51], and its high frequency could be explained by a founder effect.

### Non-syndromic hearing loss

A total of 76 and 44 probands in Queimadas and Gado Bravo, respectively, had non-syndromic HL. As summarized in **Table 2**, seven probands from Queimadas were homozygous as to the c.35delG mutation in the *GJB2* gene (7/76, 9.2%) and seven presented this mutation in heterozygosity (7/76, 9.2%). The estimated genotype frequencies in the sample of non-syndromic probands are shown in **Table 3**. This result is similar to other Brazilian studies (1.3% to 15.2%; **Table 4**). In Gado Bravo, only one homozygote with c.35delG mutation was found (1/44, 2.3%) and three were c.35delG heterozygotes (3/44, 6.8%). The frequency of c.35delG in this county was surprisingly lower than expected. The higher frequency of this mutation in Queimadas might be explained by founder effect, since six probands had a common ancestor.

Two probands had the del(*GJB6*-D13S1854) of the *GJB6* gene in heterozygosity (2/44, 4.5%) in Gado Bravo and this frequency was higher than two other Brazilian studies (1.3% and 0.5%; **Table 4**). This mutation was not detected in Queimadas. The del(*GJB6*-D13S1830) mutation was not identified in the sampled counties, as well as in other studies involving populations

of Brazilian northern and northeastern regions [36, 37]. This result is somewhat unexpected, because it is the second most frequent mutation associated with HL in southeastern populations in Brazil [30, 33-35, 38]. The c.167delT and m.1555A>G mutations were not detected in either counties.

Sequencing of the *GJB2* gene allowed identification of the p.Trp24\* (c.71G>A) in three non-syndromic probands from Gado Bravo (3/44, 6.8%). In two cases, the mutation was present in compound heterozygosity with c.35delG, and in the third case, a second mutation was not found. This mutation is found in high frequency in individuals from Asia and Romani ethnicity (Indians living as nomads in Europe commonly referred to as “Gypsies”) [53, 54]. The largest Gypsy community in Brazil is located the county of Sousa [55], far 354 km from Gado Bravo. We speculate that this mutation was introduced in Gado Bravo by this Gypsy community.

The frequencies of *DFNB1* locus mutations (cases with two pathogenic mutations) were 11.4% in Gado Bravo and 9.2% in Queimadas. This result is similar to the frequencies observed in other Brazilian studies, with frequencies ranging from 1% to 18.2% (**Table 4**). Four non-pathogenic variants, not shown in **Tables 2** and **3**, were also detected (c.-22-12C>T, c.-15C>T, c.1-6T>A and c.79G>A).

The MLPA analysis of 10 samples from probands with a single recessive mutation detected in the *DFNB1* locus and from the two patients with X-linked HL revealed no copy number variations (CNV).

Segregation studies with microsatellites mapped near to the *SLC26A4* gene were per-

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**Table 4.** Frequency of DFNB1 mutations in non-syndromic HL probands in several regions of Brazil

Genotype (DFNB1)	Campinas/ SP	São José do Rio Preto/SP	Brazil/Several Regions	São Paulo/ SP	Vitória/ ES	Belém/ PA	Marília/ SP	Monte Santo/ BA	Campinas/ SP	Gado Bravo/ PB	Queimadas/ PB
c.35delG/c.35delG	2/75 (2.7%)	5/33 (15.2%)	41/645 (6.4%)	22/300 (7.3%)	3/77 (3.9%)	1/77 (1.3%)	N	8/61 (13.1%)	14/100 (14%)	1/44 (2.3%)	7/76 (9.2%)
c.35delG/?	2/75 (2.7%)	3/33 (9.1%)	36/645 (5.6%)	5/300 (1.7%)	4/77 (5.2%)	6/77 (7.8%)	7/101 (6.9%)	1/61 (1.6%)	3/100 (3%)	3/44 (6.8%)	7/76 (9.2%)
c.35delG/Pathogenic mutation	N	N	4/645 (0.6%)**	7/300 (2.3%)	N*	N	NA	N	1/100 (1%)	2/44 (4.5%)	N
Other two pathogenic mutations in homozygosis or compound heterozygosis in the <i>GJB2</i> gene	1/75 (1.3%)	N	4/645 (0.6%)**	1/300 (0.3%)	N*	N	NA	N	1/100 (1%)	N	N
<i>GJB2</i> pathogenic mutation in heterozygosis/ del( <i>GJB6</i> -D13S1830)	NA	1/33 (3%)	4/645 (0.6%)	3/300 (1%)	1/77 (1.3%)	N	1/101 (1%)***	N	1/100 (1%)	N	N
del( <i>GJB6</i> -D13S1830)/del( <i>GJB6</i> -D13S1830)	NA	N	2/645 (0.3%)	N	N	N	N	N	N	N	N
<i>GJB2</i> pathogenic mutation in heterozygosis/ del( <i>GJB6</i> -D13S1854)	NA	NA	3/645 (0.5%)	N	1/77 (1.3%)	NA	N	N	N	2/44 (4.5%)	N
del( <i>GJB6</i> -D13S1854)/del( <i>GJB6</i> -D13S1854)	NA	NA	N	N	N	NA	N	N	N	N	N
Total of patients with two pathogenic mutations in the DFNB1 locus	3/75 (4%)	6/33 (18.2%)	58/645 (9%)	33/300 (11%)	5/77 (6.5%)	1/77 (1.3%)	1/101 (1%)	8/61 (13.1%)	17/100 (17%)	5/44 (11.4%)	7/76 (9.2%)
Reference	[31]	[30]	[32]	[33]	[34]	[36]	[35]	[37]	[38]	Present study	Present study

Table key: NP – Molecular test not performed; N – Mutation not detected. \*Only some mutations tested; \*\*Some patients selected for study by *GJB2* gene sequencing; \*\*\*Test performed only in patients with the c.35delG mutation in heterozygosis.

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**Table 5.** Numbers and classification of probands according to the most likely etiology of HL

Probably etiology	Numbers of probands in each county		
	Gado Bravo (N=55)	Queimadas (N=81)	
Genetic	AR with two mutations in DNFB1 locus	4 (7.3%)	5 (6.2%)
	AR with Usher syndrome	9 (16.4%)	1 (1.2%)
	Autosomal Recessive*	13 (23.6%)	16 (19.8%)
	Autosomal Dominant*	1 (1.8%)	11 (13.6%)
	AD or maternal inheritance*	3 (5.5%)	3 (3.7%)
	Probable X-linked inheritance	0	1 (1.2%)
Environmental	-	3 (5.4%)	12 (14.8%)
Idiopathic	-	22 (40%)	32 (39.5%)

AR – autosomal recessive; AD – autosomal dominant; \*Inferred by pedigree.

formed in 24 ARNSHL pedigrees without detected mutations in the DNFB1 locus. In 21 pedigrees, linkage to *SLC26A4* gene was excluded. Thus, three probands had all the *SLC26A4* exons completely analyzed by sequencing, but no pathogenic mutation was detected. Screening of this gene is recommended in cases with Enlarged Vestibular Aqueduct (EVA) or Mondini dysplasia [27, 56-58], and given the poverty and difficulties in accessing health services, this diagnosis was not possible in both counties. These results suggest that mutations in *SLC26A4* are not a frequent cause of ARNSHL in these counties.

### *The contribution of genetics to the etiology of hearing loss*

All probands were classified according to the most likely cause of HL and the frequencies are shown in **Table 5**. Cases with probable genetic etiology contributed approximately to half of HL probands in each county (54.6% in Gado Bravo and 45.7% in Queimadas), and, surprisingly, these frequencies are comparable to those observed in developed countries [9, 14, 59]. Both counties were selected because of previous information that HL was frequent. Thus, these figures are probably above average values for Brazil, and should not be extrapolated. Among genetic cases, AR inheritance was the most frequent in both counties (86.6% in Gado Bravo and 59.4% in Queimadas). In approximately half of ARNSHL cases from Gado Bravo a pathogenic mutation was detected (in homozygosis or compound heterozygosis) in DNFB1 locus, which is expected, according to literature [9]. However, mutations in this locus explained only 23% of the ARNSHL cases in Queimadas. One could argue that mutations in other loci

than DNFB1 and DNFB4 (*SLC26A4* gene) would be present and could explain the high frequency of AR cases in both counties, and the large frequency of DNFB1 negative cases of ARNSHL in Queimadas. A large number of cases of autosomal dominant HL cases in Queimadas (14/37, 37.8%) was found, which corresponds to a higher frequency of this type of transmission when compared to other samples (14-24%) [9].

Our survey indicated that at near 50% of the HL cases were genetically determined in the sampled counties, suggesting the need of medical and community genetics services, as it has been suggested by World Health Organization [60]. In the present study, we showed that frequency of mutations in Northeast region differs somewhat from those reported in southeastern Brazil and other populations, suggesting a specific strategy of screening for genetic HL. According to our results, the cheapest and the most appropriate strategy would be as follows: 1) PCR-RFLP to screen the c.35delG mutation; 2) Multiplex PCR to detect del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) mutations in all patients; 3) *GJB2* gene sequencing in patients with c.35delG in heterozygosis or with del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854) mutations in heterozygosis; 4) *GJB2* gene sequencing in probands with HL from pedigrees presenting with ARHL; 5) Screening of c.189C>A (p.Tyr63\*) mutation in *CLRN1* in probands with Usher syndrome from this region.

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### Disclosure of conflict of interest

None.

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