ESCOLA BAHIANA DE MEDICINA E SAÚDE PÚBLICA
CURSO DE GRADUAÇÃO EM BIOMEDICINA

JOÃO VICTOR SOUSA

FREQUÊNCIA DA MUTAÇÃO *GJB2*:c.35delG EM UMA AMOSTRA DA POPULAÇÃO DE MONTE SANTO-BA

SALVADOR
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FREQUÊNCIA DA MUTAÇÃO *GJB2*:c.35delG EM UMA AMOSTRA DA POPULAÇÃO DE MONTE SANTO-BA

Trabalho de conclusão de Curso apresentado à Escola Bahiana de Medicina e Saúde Publica como parte dos requisitos para obtenção do título de bacharel em Biomedicina.

Orientadora: Dra. Thais Ferreira Bomfim Palma.

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Esta monografia foi julgada adequada à obtenção do grau de Bacharel em Biomedicina e aprovada em sua forma final pelo Curso de Biomedicina da Escola Bahiana de Medicina e Saúde Pública.

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Dra. Thaís Ferreira Bomfim Palma
Laboratório de Imunologia e Biologia Molecular (Labimuno), Instituto de Ciências da Saúde (ICS), Universidade Federal da Bahia (UFBA)

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Dra. Taisa Manuela Bonfim Machado Lopes
Laboratório de Imunologia e Biologia Molecular (Labimuno), Instituto de Ciências da Saúde (ICS), Universidade Federal da Bahia (UFBA)

_____

Dra. ThessikaHialaAlmeia Araújo
Escola Bahiana de Medicina e Saúde Pública

SUMÁRIO

GJB2:c.35delG mutation frequency in Monte Santo BA Population sample

João Victor Sousa[a,b], Kiyoko Abe Sandes[b], Gabrielle Novais Manzoli[c], Angelina Xavier Acosta[d], Thais Ferreira Bomfim Palma[b,*]

[a]Bahiana School of Medicine and Public Health, Salvador, Bahia,.Brazil

[b]Immunology and Molecular Biology Lab (LABIMUNO), Institute of Health Sciences (ICS), Federal University of Bahia (UFBA), Salvador, Bahia, Brazil

[c]Gonçalo Muniz Research Center (CPqGM), Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Bahia, Brazil

[d] School of medicine at the Federal University of Bahia (UFBA), Salvador, Bahia, Brazil)

* Corresponding author at: Immunology and Molecular Biology Lab, Institute of Health Sciences, Federal University of Bahia, Av. Reitor Miguel Calmon 1272, Vale Canela, CEP 40110-902, Salvador, Bahia, Brazil. Tel.: +55 (071)3283-8935
E-mail address: thaisbomfim@gmail.com

Declarations of interest: none.

Abstract:

Objective: The *GJB2*:c.35delG mutation is very common among the individuals with non-syndromic hearing impairment at Monte Santo City of countryside state of Bahia, which makes necessary the study of the frequency of the mutation on the general population. Methods: This study investigated 269 non-deaf individuals for the *GJB2*:c.35delG variant through DNA Sanger sequencing. Results: 9 out of the 269 individuals were heterozygous carriers for the mutations, resulting in 3,35% of the genotypic frequency. Discussion: Other studies corroborates with the frequency found, due the European ancestry of the city and the high frequency of endogamic marriage. Conclusions: Beyond have a homozygous mutant high frequency among NSHI individuals, Monte Santo also has an elevated frequency of heterozygous carriers of this variant.

Keywords: Non-syndromic hearing impairment, *GJB2*, c.35delG, Connexin26, Genetics, Monte Santo.

1.Introduction

Hearing impairment (HI) is characterized by the decrease or inability of the auditory system transmit sound. Considered the most common sensory deficit, once it affects 2: 1000 live births. HI can be classified as prelingual, when the deficiency manifests itself to be severe or profound before the oral language acquisition. It is also known that 2 to 4 children in a thousand will become hearing impaired before adulthood (post-lingual HI) [1–3].

HI also can be classified by the type: conductive, central, mixed or sensorineural, due the affected anatomical segment that prevents the sound transmission; by the severity: mild, moderate, severe or profound, depending on the threshold of decibels that the affected individual can hear; by the progression over the time, being progressive or non-progressive; and by the laterality, if both ears are affected or not[2,4,5].

HI can be an environmental and / or genetic etiology [2,5]. Genetic cases can be divided into syndromic, when involves other different signs and symptoms besides deafness, or an isolated HI, known as Non-syndromic Hearing Impairment (NSHI), which is commonly associated with the sensorineural type hearing loss and affects prelingual age individuals [2,5,6].

More than 80 genes have already been associated with NSHI, of these, more than 50 have autosomal recessive inheritance pattern mutations[1,7,8]. In this group is included the GJB2 gene, in which more than 100 pathogenic mutations associated with HI have already been described. Depending on the population studied, it was observed that pathogenic mutations on the *GJB2* gene are responsible for up to 50% of NSHI cases [9].

The *GJB2* gene was the first gene associated with non-syndromic genetic HI with autosomal recessive inheritance pattern described by Kelsell et al. (1997) [10]. The HI caused by mutations in the GJB2 gene is characterized by being prelingual, profound, bilateral and non-progressive [11]. This gene codes the connexin 26 (Cx26) protein, which is involved in the formation of gap junctions and plays a key role in controlling the flow of potassium ions between the ciliated cells and the endolymph in the cochlea, playing a crucial role in hearing, transforming the sound vibration in an electrical impulse that is transmitted to the cochlear nerve [10,12].

One of the mutations in the GJB2 gene first associated with NSHD was GJB2: c.35delG (rs80338939) described in European and Pakistani populations [13,14].

This variant is considered to be one of the most frequent in Caucasian populations, accounting for about 70% of NSHI cases [15,16]. In Brazil, the variant frequency varies between 0,8% at Northeast region, 1,17% at South region, 1, 56% at Southeast region, and 2,12% at North region [12].

In Monte Santo, a city of countryside state of Bahia with 52338 inhabitants [13]that has high frequency of consanguineous marriage, low immigration rate, subdivision of the population in smaller villages and farms, and high frequency of some genetic disease, including NSHI. 152 individuals were identified with the condition, witch 24,7% had the *GJB2*:c.35delG mutation identified as cause of the hearing loss [14–17].

This study aims to investigate the frequency of the *GJB2*:c.35delG mutation in a general Monte Santo population sample.

2.Subjects and Methods

2.1.Population Sample

This study is a subproject from the main project called Census “Genetics in Sertão”: Classic and Molecular Epidemiology about Genetic Diseases in the City of Monte Santo-BA (Certificate of Presentation for Ethical Consideration number 17885313.1.1001.0049 and Feedback Number 379.010).To carry out the Monte Santo census, a randomized draw of 2.5% of the 13,000 families living in this CITY was carried out, followed by another draw of 1 member from each selected family, according to the distribution of the population according to the performance of the Family health teams of the city. All the 325 sorted individuals were invited to participate and to the ones that accepted, it was signed the Term of Consent and collected 4ml of peripherical blood in EDTA tube for each participant, besides an ancestry questionnaire.

In this study was analyzed 269 healthy, non-deaf individuals coming from the sortition, for the *GJB2*:c.35delG (rs80338939) mutation investigation.

2.2.Molecular Analyses

The genomic DNA was extracted from the collected peripherical blood during the expeditions to the city of Monte Santo-BA, following the kit producer recommendations (Extraction Kit Mini Spin Plus – Biometrix, Biopur),then applied in

2,5% agarose gel and submitted to the electrophoresis process to verify the sample integrity.

After the genetic material was obtained, it was made a Polimerase Chain Reaction (PCR)[18]to amplify a 332 base pairs (BP) fragment of the second exon of the *GJB2* gene.

The amplification reaction was realized starting with 100ng of genomic DNA, using the *Forward* (5' – TCTTTTCCAGAGCAAACCGC– 3') and *Reverse* (5' – GCTGGTGGAGTGTTTGTTCACACCCGC – 3') primers. The cycle used consisted of a starting denaturation: 95°C for 5 minutes, followed by 35 repetitions of denaturation, primer annellation and strands extension: 95°C-1 minute, 62°C-1 minute, and 72°C-2 minutes; to finish, the PCR, it was realized a final extension cycle of 72°C for 10 minutes.

The PCR product was also submitted to an electrophoresis process in a 2,5% agarose gel to be confirm that the reaction worked, and the region of interest was amplified, comparing to a molecular weight ladder.

The PCR product was then purified using commercial kits (Wizard SV Gel and PCR Clean-Up System, Promega) and sequenced by Sanger methodology [19]. The sequencing reaction was precipitated with 80µl of isopropanol 70% followed by resuspension with 10µl of formamide Hi-Di (Thermo Fisher Scientific), and desaturated at 95°C for 5 minutes followed by thermic shock in ice for 2 minutes for then be analyzed in capillary electrophoresis in automatic sequencer 3130xl of Applied Biosystems® from Thermo Fisher Cientific.

2.3.Data analyses

The data obtained through capillary electrophoresis was analyzed in BioEdit version 7.0.5.3 software for the variant identification. The results also was analyzed on GENEPOP on the WEB version 4.2 calculate the Hardy-Weinberg (H-W) Test p-value.

3.Results

The sequencing of*GJB2*exon 2 in general population of Monte Santo-BA showed9 heterozygous for the *GJB2*:c.35delG mutation and 260 wild-type homozygous. As expected none mutant homozygous was found.

Genotype	Number of individuals (N)	Genotypic Frequency (%)
Wild-type homozygous	260	96,65
Heterozygous	9	3,35
Total	269	100

Table 1

Absolute number of individuals and Genotypic Frequency of Wild-type homozygous and *GJB2*:c.35delG heterozygous.

The c.35delG variant allelic frequency was 1,67%. The p-value for the H-W test wasn't statistically significant (p-value: 1,0), suggesting that the population is in equilibrium with H-W.

4.Discussion

The city of Monte Santo was described by have a higher frequency of NSHI than Bahia and Brazil (0,29, 0,17 and 0,18 respectively)[20]. Considering the *GJB2*:c.35delG the main mutation causing NSHI, the frequency of this variant in Monte Santo affected individuals (24,4%) shows higher frequencies than other Brazilians cities and even other countries (Table 2).

Location	Frequency (%)
Monte Santo	24,1
Espírito Santo	3,9
São Paulo	7,3
Brazil (General)	6,5
Mexico	2,6
France	6,3
Italy (Northern)	16,9
Italy	30,2
Croatia	25,4
Bulgaria (Bulgarian and Turkish)	39,2
Altai Republic (all Russian)	9,2
Turkey	5,3
Iran (Azerbaijani)	15,3

Iran (Ardabil)	18,0
Iran	10,0
China (Han)	0,8
China	0,1
Saudi Arabia	6,4
South Africa (Limpopo)	0,0

Table 2

Frequency of the *GJB2*:c.35delG homozygous individuals among NSHI (Adapted from Manzoli (2013) [20]

In this study was found a frequency of the *GJB2*:c.35delG mutation of 1,67%. This result corroborates with other studies that evaluated populations without NSHI, like Sartorato (2001) in São Paulo, with 620 random new-borns that identifies that 0,97% were heterozygous for the variant[21].

In Oliveira's (2007) study, was descript the frequency of the variant *GJB2*:c.35delG in different Brazilian regions, by city of study, was described, and in some of them the mutation was not found, as in Sorocaba-SP, in others, frequency ranged from 0.67% (Porto Alegre - RS) to 4.16% (Espírito Santo do Pinhal - SP) [17].

Gasparini (2000) evaluates the frequency of this variant in a control sample in 19 different European population, the frequency varies between 0% (United Kingdom) to 3,38% (Italy-Sardinia)[22]. In that study can be observed the heterogeneity in *GJB2*:c.35delG distribution when related to geographic region, emphasizing the variant presence in Portugal, Spain and Italy, countries that population had great importance during the formation process of Brazilian population.

The *GJB2*:c.35delG variant is very common in Europe and Gasparini (2000) study suggests one single origin for the mutation on some point of Europe or Middle East [22].

Genetic ancestry was evaluated in the Monte Santo City through the study of biparental markers informers of ancestry that shown a frequency differential of more than 30% between geographically distinct population, as well as evaluating uniparental markers (Y chromosome and mitochondrial DNA). On that study, Machado (2012) described the ancestry of Monte Santo-BA predominantly European

(61%, in HI individuals) Between the homozygous individuals for the mutation *GJB2*:c.35delG there is an even higher European contribution (82,9%)[16].

Oliveira (2004) study, evaluates the genotypic frequency in Asian, European and African Brazilian descendants, founds the frequencies 0,0%, 2,0% and 1,0% respectively for *the GJB2*:c.35delG variant, reaffirming the importance of European ancestry in the population history[23].

Although the absolute number of carriers of the variant was low, the genotypic frequency of mutant homozygous among NSHI could be explain by the high frequency of endogamic marriage, as described by Machado (2012), increasing the chances to the next generation inherit both mutant alleles[16].

The p-value being 1,0 shows the population is in H-W equilibrium and will maintain stable if won't suffer any evolutionary pressure. As seen in Machado (2012) study, Monte Santo is a city with low immigration tax, been the most part coming from the borderer cities[16], favoring the continuity of the variation frequency on the population.

It was described that individuals with HI caused by *GJB2*:c.35delGand others *GJB2* variations may have a better hearing performance with early childhood cochlear implants[24,25]. Individuals that don't undergo implantation until adult age don't usual respond to words and phonemes[25].

The *GJB2*:c.35delG mutation could be added to the newborn screening program in the city as it recently happened to the arylsulfatase B (*ARSB*) gene, which variations may lead to Mucopolysaccharidosis VI phenotype, alongside the screening for other disease like Phenylketonuria, hypothyroidism and hemoglobin disorders [15,26]. This newborn screening could help to early identify new cases of hearing-impaired individuals due to the variant and give the proper genetic counseling to them and their families [27,28].

Testing for known the etiology of HI is an essential step to the treatment, such early cochlear implants in children. The GJB2:c.35delG mutation isn't the only variation responsible for NSHI on Monte Santo, although being one of the most important, so testing for this and other variants in neonatal screening is essential for improve the early diagnosis and so the life quality of hearing impaired individuals.

This is the first study to describe the *GJB2*:c.35delG frequency in a representative sample of the Monte Santo's general population, which may help describe the risk for the population and future generations.

5. Conclusion

Given its characteristics, Monte Santo is described by high frequencies of genetic diseases as NSHI. Previous studies already indicate that *GJB2*:c.35delG variant was one of the main between the affected individuals, but the present study indicates that the frequency of carries is high in general population, which means that the risk is extended to future generations. Other studies like this become necessary in population with characteristics and history similar to Monte Santo, primarily the borderer cities to compare the profile of the populations.

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2.Proposta de submissão

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Blake Papsin, MD, MSc, FRCSC, FACS, FAAP, The Hospital for Sick Children (SickKids), Toronto, Ontario, Canada
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[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
Reference to a dataset:
[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/xwj98nb39r.1.

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3.Appendix

Location	Frequency (%)	Study
Monte Santo-BA	3,35	This Study
Denmark	2,1	[22]
Norway	0,53	[22]
Estonia	4,44	[22]
United Kingdom	0,0	[22]
Germany	2,0	[22]
Belgium	0,53	[22]
Holland	2,25	[22]
France (Brittany)	1,04	[22]
France	0,5	[22]
Czech Republic	2,05	[22]
Slovenia	0,54	[22]
Bulgaria	0,63	[22]
Portugal	2,22	[22]
Spain	2,5	[22]
Italy	3,12	[22]
Italy (Sardinia)	3,38	[22]
Malta	2,78	[22]
Greece	3,03	[22]
Turkey	2,67	[22]
São Paulo (Not Stated)	0,97	[21]
Belém-PA	2,13	[12]
Sorocaba-SP	0,0	[12]
São José do Rio Preto-SP	2,22	[12]
Espírito Santo do Pinhal-SP	4,16	[12]
Jundiaí-SP	1,0	[12]
Patos de Minas-MG	1,0	[12]
Porto Alegre-RS	0,67	[12]
Joinville-SC	1,1	[12]
Curitiba-PR	2,0	[12]
Fortaleza-CE	0,81	[12]

Appendix 1

Genotypic frequency of the *GJB2*:c.35delG heterozygous individuals around Europe and Brazil.