


# Exome Sequencing Identifies a Novel Nonsense Mutation of *MYO6* as the Cause of Deafness in a Brazilian Family

Juliana Sampaio-Silva<sup>1</sup>, Ana Carla Batisso<sup>1</sup>, Rafaela Jesus-Santos<sup>1</sup>, Osório Abath-Neto<sup>2</sup>, Luciano Cesar Scarpelli<sup>3</sup>, Patricia Yoshie Nishimura<sup>3</sup>, Layla Testa Galindo<sup>3</sup>, Ricardo Ferreira Bento<sup>1</sup>, Jeanne Oiticica<sup>1</sup> and Karina Lezirovitz<sup>1\*</sup> 

<sup>1</sup>Laboratório de Otorrinolaringologia/LIM32, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil

<sup>2</sup>Departamento de Neurologia, Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, Brasil

<sup>3</sup>Setor de Biologia Molecular, Grupo DASA – Diagnósticos da América, Barueri, SP, Brasil

## Summary

We investigated 313 unrelated subjects who presented with hearing loss to identify the novel genetic causes of this condition in Brazil. Causative *GJB2/GJB6* mutations were found in 12.7% of the patients. Among the familial cases (100/313), four were selected for exome sequencing. In one case, two novel heterozygous variants were found and were predicted to be pathogenic based on bioinformatics tools, that is, p.Ser906\* (*MYO6*) and p.Arg42Cys (*GJB3*). We confirmed that this nonsense *MYO6* mutation segregated with deafness in this family. Only the proband and her unaffected mother exhibited the *GJB3* mutation, which is in the same amino acid of a known *Erythrokeratoderma variabilis* mutation. None of the patients exhibited this skin disease, but the proband exhibited a more severe hearing loss. Hence, the *GJB3* mutation was considered to be a variant of uncertain significance. In conclusion, we described a novel nonsense *MYO6* mutation that was responsible for the hearing loss in a Brazilian family. This mutation resides in the neck domain of myosin-VI after the motor domain. Thus, our data give further support for genotype-phenotype correlations, which state that when the motor domain of the protein is functioning, the hearing loss is milder and has a later onset. The three remaining families without mutations in the known genes suggest that there are still deafness genes to be revealed.

Keywords: Hearing loss, *MYO6* gene, Exome sequencing, *GJB3* gene

## Introduction

Hearing loss (HL) or deafness is one of the most common sensorineural defects in humans. Nonsyndromic hearing loss (NSHL) is highly heterogeneous in its clinical presentation (i.e., age at onset, progression, and audiological characteristics), pattern of inheritance and underlying genetic causes (Smith et al., 2005). With the progress of prenatal and early postnatal health attention, the majority of occurrences of HL in prelingual children are of genetic etiology (50–60%) (Mehta et al., 2016). With recent advances in molecular genetics, our understanding of the pathogenesis of sensorineural HL has

greatly expanded, and to date, mutations in more than 100 genes involved in autosomal dominant or autosomal recessive NSHL have been identified (Van Camp & Smith, 03/2017). The identification of causative genes for hereditary NSHL is important for decisions about the treatment modalities and the counseling of patients.

Except for the relatively common genes *GJB2* and *GJB6*, the most reported mutations are present in only a single or a few families (Vona et al., 2014; Nishio & Usami, 2015; Zazo-Seco et al., 2017), and thus the genetic causes remain unknown for the majority of deaf individuals. The etiologic contributions of the less commonly screened deafness genes in particular have yet to be investigated systematically in many populations. Due to the large number and presumably low mutation frequencies of those genes, it would be highly expensive and time-consuming to address this issue with conventional Sanger sequencing. A growing number of research and clinical diagnostic laboratories are successfully using

\*Corresponding author: KARINA LEZIROVITZ, PhD, Otolaryngology Lab/LIM32- University of São Paulo School of Medicine, Av. Dr. Arnaldo, 455 2nd Floor, Room 2209, São Paulo – SP – Brasil. Zip Code: 01246-000. Tel: +55 11 3061-7166; E-mail: lezi.karina@gmail.com

next-generation sequencing technologies for gene/variant identification (Schnekenberg et al., 2013). With the genomic capturing of a wide selection of known deafness genes, it is possible to screen these genes in a high-throughput manner by next-generation sequencing.

In this study, we present the results of the molecular analysis from a family that segregated with autosomal dominant NSHL. This family was one of four familial cases that was selected for exome sequencing from a large multiethnic group consisting of 313 unrelated Brazilian subjects with hearing impairments.

## Patients and Methods

### Patients data and family ascertainment

A total of 313 Brazilian unrelated subjects from all over the country who presented with hearing impairment were referred to the Genetic Deafness Counseling Unit of the Otolaryngology Department (Clinics Hospital of University of Sao Paulo School of Medicine). This study was approved by the Ethics Committee for Analysis of Research Projects from the University of Sao Paulo School of Medicine. Written informed consent was obtained from all hearing-impaired individuals or their legal guardians, their relatives, and control individuals. We did not exclude patients with suspected environmental causes or syndromic features from the analysis. Approximately 31% of our cohort (100/313) were familial cases, and four of them in which the segregation pattern was compatible with autosomal dominant inheritance were selected for exome sequencing. All four familial cases had at least three documented affected subjects who agreed to participate in the study.

### Clinical evaluation

Pure tone audiometry was performed to test for air conduction (250–8000 Hz) and bone conduction (250–4000 Hz). Syndromic features were assessed after physical examinations and complete anamneses. No symptoms of vestibular dysfunction were reported. Malformations of the inner ear were examined by high-resolution computed tomography of the temporal bones in the proband.

### Molecular analysis

Genomic DNA was extracted with commercial kits from the peripheral blood leukocytes. All subjects were screened for the following: mutations in the coding region of the *GJB2* gene by conventional Sanger sequencing, the  $\Delta$ (*GJB6*-D13S1830) and  $\Delta$ (*GJB6*-D13S1854) deletions by multiplex PCR (del

Castillo et al., 2005), and the m.1555A>G mitochondrial mutation (MT-RNR1) by restriction enzyme digestion analysis (Estivill et al., 1998).

### Exome sequencing

The DNA qualities and quantities were verified with a Nanodrop spectrometer (Nanodrop Technologies, Wilmington, DE, USA) and a Qubit fluorometer (Life Technologies, Grand Island, NY, USA). Amplicon libraries were prepared according to the manufacturer's instructions using the Ion PI Library kit plus an Ion PI Ampliseq Exome RDY 1 × 8 (Applied Biosystems, ThermoFisher Scientific Inc., Waltham, MA, USA), and an Ion PI Hi-Q OT2 200 kit was used for template preparation. Sequencing was conducted with an Ion PI Hi-Q Sequencing 200 kit and an Ion PI Chip v3 in a Proton Semiconductor Sequencer–Ion Torrent (Life Technologies, Thermo Fisher Scientific Inc.).

### Base call and data analysis

Variant filtering was performed as follows. First, the VCF files were parsed into a database management system (MySQL, <http://www.mysql.com/>) in which we integrated the cohort- and batch-wise variant annotations. We then excluded the variants that were expected to have a low pathogenicity (intergenic, intronic located away from the splice sites, and synonymous variants that did not interfere with the splice sites). The next step was to remove variants with minor allele frequencies (MAFs) in the Exome Aggregate Consortium (ExAC) database greater than 0.5% as well as the variants with a MAF greater than 0.5% in a local variant database to account for the local population-specific variants or systematic errors in sequencing or mapping methods. Finally, we filtered the variants found in the genes implicated in hereditary hearing loss as listed in <http://hereditaryhearingloss.org/> (Van Camp & Smith, 2017) and ranked them according to the presumed inheritance patterns in each family.

### Sanger sequencing

Sanger sequencing was performed to screen for *GJB2* mutations, to validate the exome results and to perform segregation analysis of the candidate variants. The primers were designed with Primer3 ([bioinfo.ut.ee/primer3-0.4.0/](http://bioinfo.ut.ee/primer3-0.4.0/)) using the following reference sequences: the *MYO6* gene (NM\_004999), the *GJB3* gene (NM\_024009), and the *OTOA* gene (NM\_144672). After purification of the PCR products with the enzymes Exonuclease I and FastAP Thermoinsensitive Alkaline Phosphatase (Fermentas, ThermoFisher Scientific Inc., Waltham, MA), they were sequenced with an

ABI Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, ThermoFisher Scientific Inc., Waltham, MA), purified with ethanol/sodium acetate/EDTA precipitation and analyzed in an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific Inc., Waltham, MA).

### Conservation and pathogenicity

Five bioinformatics tools were used for pathogenicity prediction (PolyPhen2, SIFT, MutationTaster, VEP, and Provean). The evolutionary conservation of each variant position was measured with PhyloP (Siepel et al., 2005) and PhastCons found in the MutationTaster analysis. The missense variant was also evaluated for potential pathogenicity using protein prediction tools, such as SNPs3D (<http://www.snps3d.org>), PhD-SNP (<http://snps.biofold.org/phd-snp/phd-snp.html>), and SOSUI ([http://harrier.nagahama-i-bio.ac.jp/sosui/cgi-bin/adv\\_sosui.cgi](http://harrier.nagahama-i-bio.ac.jp/sosui/cgi-bin/adv_sosui.cgi)). We used the standards and guidelines from Richards et al. (2015) for the interpretation of the sequence variants regarding the evidence for pathogenic or benign classification status.

### Variant databases

All variants were searched in the Deafness Variation Database ([deafnessvariationdatabase.com](http://deafnessvariationdatabase.com)), which compiles pathogenic and non-pathogenic variants of known deafness genes, the ExAC browser ([exac.broadinstitute.org/](http://exac.broadinstitute.org/)), the 1000 Genome Project Database (<http://www.1000genomes.org/node/home>), and the ABraOM (Brazilian genomic variants, <http://abraom.ib.usp.br>, 03/2017). The ABraOM comprises the exome variants from 609 elderly healthy individuals from the Brazilian population.

## Results

We molecularly investigated 313 unrelated Brazilian subjects who presented with hearing impairment. Among these subjects, 8% had syndromic features, and the remaining subjects were nonsyndromic. From the group of 313 subjects, 100 were familial cases (31%). From among the familial cases, four were selected for exome sequencing because they had at least three documented affected individuals who agreed to participate in the study. All participants exhibited postlingual onset and autosomal dominant inheritance as the most likely inheritance mechanism. After exome sequencing, variants in known deafness genes were found in only one of the four families. This family had 13 affected individuals in four generations who supported an autosomal dominant mode of inheritance (Fig. 1).

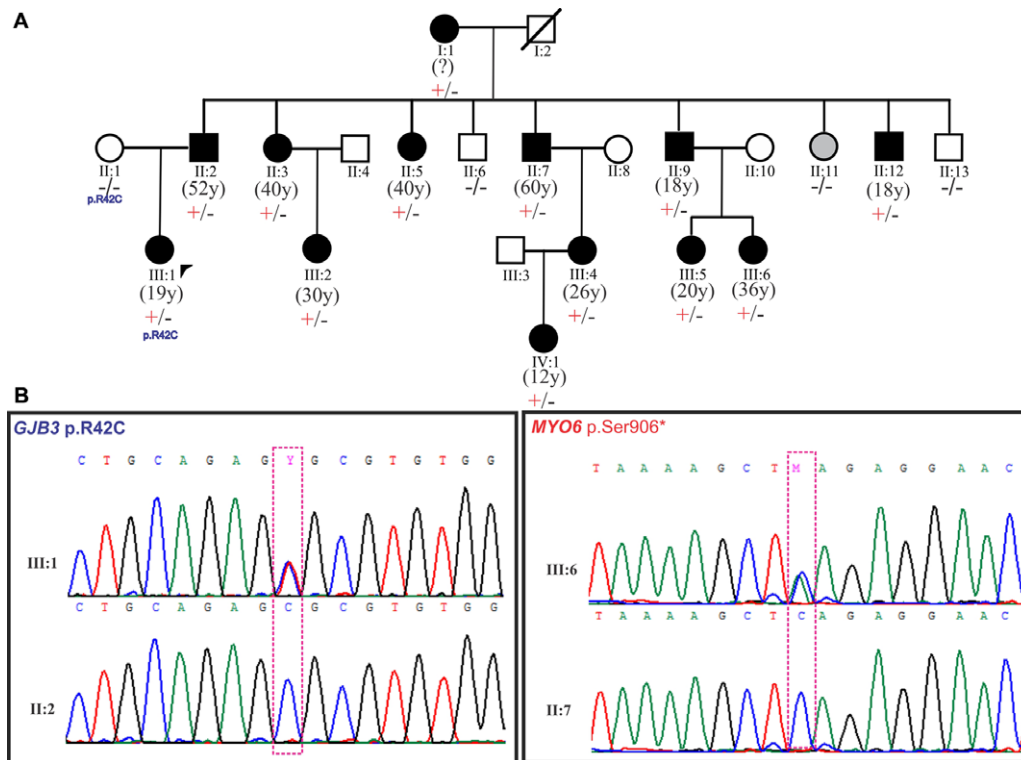
### Clinical description

Computed tomography scan analyses of the proband ruled out the presence of inner ear malformations. Audiograms were available from 12 affected individuals, including one individual with a distinct clinical phenotype (II:11) and two unaffected family members (Fig. 2). We divided the audiograms into two figures based on the age at assessment, that is, 19–37 years (Fig. 2A) and 55–87 years (Fig. 2A). The hearing loss was sensorineural and bilateral, except for subject II:1. At the onset, the hearing impairment mainly affected the high frequencies with a downsloping audiometric configuration in all family members. The referred age of onset varied between 12 years (IV:1) and 60 years (II:7). Progression was well documented in the proband (audiograms III:1–19y and III:32y, Fig. 2A), who exhibited asymmetrical hearing loss that was profound for high frequencies in the right ear by the age of 32 years. The remaining audiograms from three family members aged in their 30s revealed mild hearing loss, whereas the other seven audiograms from three family members aged between 55 and 65 years old revealed moderate hearing loss that also indicated progressive hearing loss. Thus, the proband's phenotype was more severe compared with her cousins' of the same age. The most severe hearing impairment was demonstrated by the oldest affected subject at 87 years of age who presented with severe impairment in the low-mid frequencies and profound impairment in the high frequencies. A medical history revealed that the proband had rheumatic fever at 18 years of age, or approximately 1 year before noticing the hearing impairment. The other affected individuals did not refer to any disease prior to the hearing loss onset. To date, there have been no reports on the association between rheumatic fever and hearing loss (Schubert et al., 2001).

Patient II:11 suffered a tympanic perforation during childhood because of a tonsil infection. A tonsillectomy was performed at 12 years old, and a tympanoplasty was performed at 16 years old. Because she experienced recurrent ear infections that resulted in unilateral mixed hearing loss (Fig. 2C), her hearing loss was considered a phenocopy.

### Genetic findings

Mutations in the *GJB2/GJB6* genes explained 12.7% of the 313 cases, including one syndromic subject who presented with palmoplantar keratoderma. Considering the familial cases, regardless of which was the most likely inheritance mechanism, *GJB2/GJB6* causative mutations explained 13%, and among the sporadic cases, 12.2%. Among the biallelic *GJB2/GJB6* subjects, 65% were c.35delG mutation homozygotes, and 24% were compound heterozygotes for this mutation. The p.Trp24\* mutation was the second most common mutation in *GJB2*, with one homozygote



**Figure 1** (A) Pedigree of the family segregating with autosomal dominant bilateral sensorineural hearing loss. The referred ages at onset are indicated inside the brackets. The +/- indicates which individuals had the *MYO6* mutation. The grey shading of symbol II:11 indicates that she presented unilateral mixed hearing loss and her hearing loss was considered a phenocopy. (B) Electropherograms of the *GJB3* and *MYO6* mutations showing the novel variants identified in the present study. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

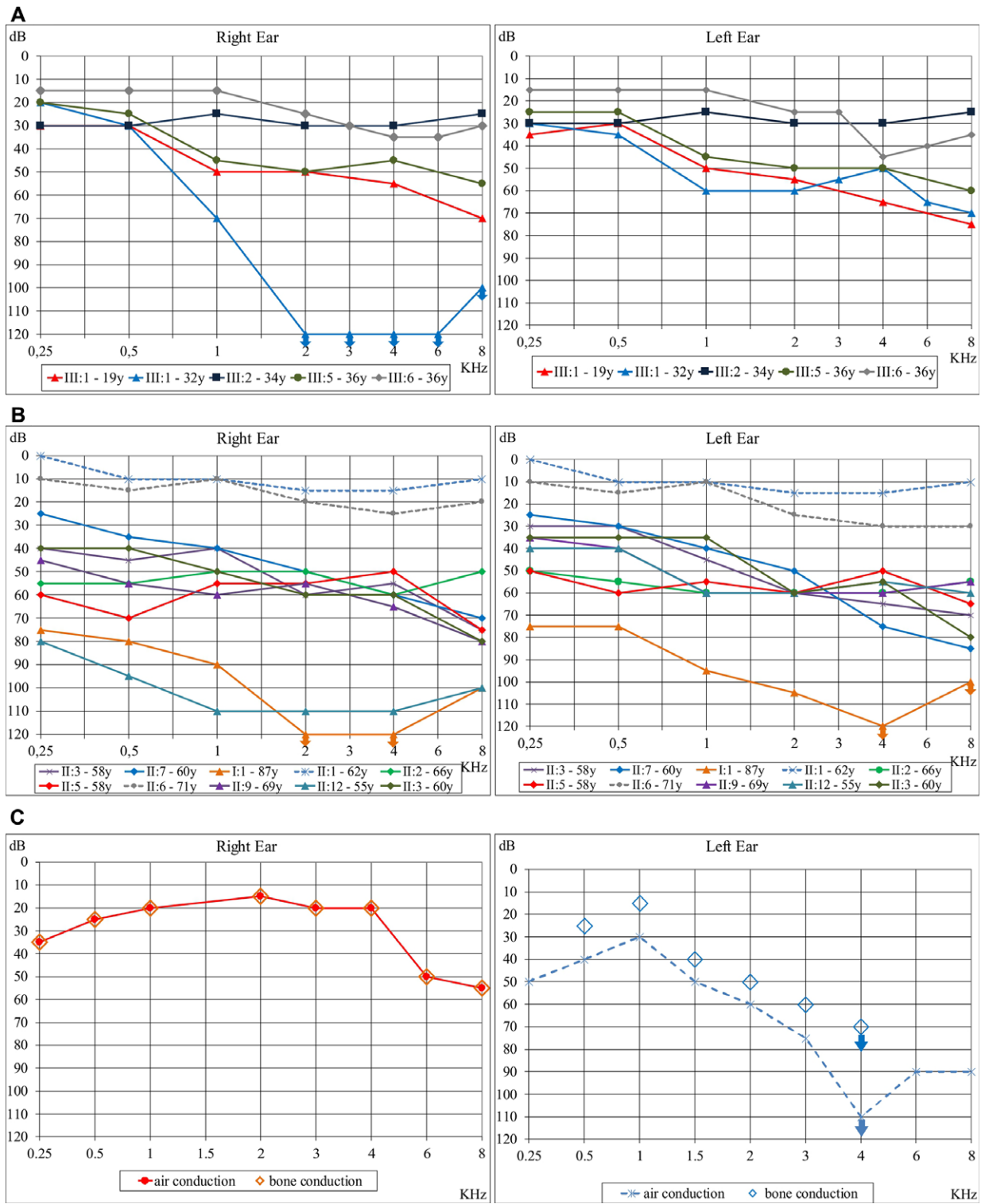
(~3% of the sample) and 11% compound heterozygotes [c.35delG/ p.Trp24\*, p.Trp24\*/p.Arg184Pro, and p.Trp24\*/p.Val95Met]. We found three cases of *GJB6* deletions in combination with the c.35delG mutation, including two with delGJB6–D13S1830 and one with delGJB6–D13S1854 mutations. Six subjects were monoallelic for c.35delG. The m.1555A>G mitochondrial mutation was present in 1% (3/313).

In family 1, after exome sequencing, we detected four variants in three different genes that had already been associated with deafness: the nonsense heterozygous variant c.2717C>A (p.Ser906\*, NM\_004999) in exon 26 of the *MYO6* gene (DFNA22/DFNB37), the missense heterozygous variant c.124C>T (p.Arg42Cys, NM\_024009) in exon 2 of the *GJB3* gene (DFNA2B), and two variants in the *OTOA* gene (DFNB22) including one nonsense [c.2401G>T, p.Glu801\*, rs200988634, NM\_144672] and one missense [c.2395A>C, p.Thr799Pro, rs464696, NM\_144672] gene both in exon 21.

Regarding the *OTOA* gene, the p.Thr799Pro variant was considered a polymorphism/neutral/tolerated by

bioinformatic tools (MutationTaster, Sift, Provean) and benign by the Deafness Variation Database, but the p.Glu801\* variant was considered to be disease-causing by MutationTaster, because it was a nonsense mutation, but was listed as benign by the Deafness Variation Database. Additionally, both *OTOA* variants were detected in the ABraOM database from the Brazilian population with a frequency of approximately 2–3% as well as in ExAC [p.Thr799Pro – 0.17% and p.Glu801\* – 0.12%]. Moreover, segregation analysis ruled out *OTOA* gene as the causative gene because mutations in this gene cause autosomal recessive deafness, and all affected and unaffected family members, including the proband's mother and one control individual with normal hearing, have both variants, suggesting that they might be in the same allele (*cis*).

Both the *GJB3* and *MYO6* variants described herein have not been reported so far in the Deafness Variation Database, ExAC, 1000 Genomes, or ABraOM. The *MYO6* mutation, p.Ser906\*, was predicted to be pathogenic by all bioinformatic programs (MutationTaster, VEP) as well as the *GJB3* mutation, p.Arg42Cys (MutationTaster, Sift, PolyPhen2, Provean).



**Figure 2** Audiograms of family members: (A) The individuals’ ages when tested were between 19 years and 37 years. (B) The individuals’ ages when tested were between 55 years to 87 years, and the audiograms that were within the normal range are represented with dotted lines. (C) Individual II:12 who exhibited a unilateral mixed hearing loss due to a tympanic perforation. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

The *MYO6* variant changed a highly conserved nucleotide across vertebrate species (PhyloP = 5.718 and PhastCons = 1), whereas the *GJB3* variant changed a nucleotide that was also conserved but with weaker scores (PhyloP = 0.692 and PhastCons = 0.977). The p.Arg42Cys variant was also predicted to be deleterious by protein prediction tools: with a SNPs3D score of -0.2; a disease 8 by PhD-SNP; and the generation of an increased average hydrophobicity of the protein from 0.047778 to 0.073704, as calculated by SOSUI. We confirmed that the *MYO6* mutation (p.Ser906\*) segregated with deafness within the pedigree, as illustrated in Figure 1, whereas the *GJB3* variant was confirmed to be heterozygous in the proband (Figure 1) as well as in her unaffected mother but was not detected in any other affected family member.

## Discussion

Pathogenic mutations in *GJB2/GJB6* exhibited a similar frequency (~12%) in our sample when compared to a study that was previously conducted in Brazilian patients who were also from the city of São Paulo (Batissoco et al., 2009). In the city of Campinas, the frequency of *GJB2/GJB6* mutations were found to be higher (22%) by Oliveira et al. (2002). In contrast to the present study and the study from Batissoco et al. (2009), these authors excluded from the analysis syndromic cases and cases with possible environmental causes. If both cases were excluded from our sample, *GJB2/GJB6* mutations would be responsible for 16% of the cases. Oliveira et al. (2002) encountered 50% of her familial cases with *GJB2* mutations, but only 11.5% were sporadic cases. We found similar frequencies in both the familial and sporadic groups of approximately 12%. We hypothesize that the small sample size and a possible ascertained bias toward prelingual cases and/or familial cases with autosomal recessive inheritance in Oliveira et al.'s study might explain the different *GJB2* mutation prevalence between their study and the present study. The m.1555A>G mitochondrial mutation was less frequent in our sample (1%) than in the Brazilian studies from Abreu-Silva et al. (2006) (i.e., 2%) and from Alves et al. (2016).

Exome sequencing of four familial cases of autosomal dominant hearing loss identified two potentially pathogenic variants in two different deafness genes (i.e., *MYO6* and *GJB3*) in one case. The *MYO6* mutation segregated with deafness within the family, but the *GJB3* was only harbored by the proband and her unaffected mother. Both variants resided in conserved positions based on the PhyloP and PhastCons, although the *MYO6* variant had much higher values. Mutations in the *MYO6* and *GJB3* genes have previously been reported in families that segregate autosomal dominant and autosomal recessive hearing loss (Tables 1 and 2). Mutations in *MYO6* have also been associated with an inherited form of hyper-

trophic cardiomyopathy (Mohiddin et al., 2004; Hegan et al., 2015), whereas mutations in *GJB3* are associated with a skin disease known as *Erythrokeratoderma variabilis* (Richard et al., 1998).

The *MYO6* gene has 35 exons (NM\_004999) with 40 pathogenic mutations that have been described to date. This gene codifies the myosin VI protein with 1285 amino acids, which is essential for the development, structural integrity and appropriate functioning of the inner ear hair cells, and for moving and transporting cargo toward the minus end of actin filaments (Wells et al., 1999; Sweeney & Houdusse, 2007). Myosin VI localizes to the cuticular plate of hair cells and is important for providing protection by restraining mechanical forces (Avraham et al., 1995; Hasson et al., 1997; Self et al., 1999). The protein consists of a motor domain containing an ATP- and an actin-binding site, followed by a neck domain and a globular tail, which interacts with other proteins (Sweeney & Houdusse, 2007). One hypothesis to explain the genotype–phenotype correlation for the dominant *versus* recessive mutations in the *MYO6* gene was proposed by Topsakal et al. (2009). If the protein motor domain is not affected and functions normally the hearing loss is milder and has a later onset. Indeed, the *MYO6* mutation reported here only affects the neck domain and keeps the motor domain intact. Our family resembles the DAN and BEL2 families analyzed by Topsakal et al. (2009) as well as the family described by Yang et al. (2013) in which the mutations leading to premature stop codons after the motor domain caused autosomal dominant late-onset hearing loss. In addition, this mutation is predicted (MutationTaster) to lead to mRNA degradation of some or all of the mRNA from the mutated alleles by NMD and thus result in less protein in affected individuals and hearing loss.

The *GJB3* gene encodes connexin 31 (Cx31), which is also known as the gap junction beta-3 protein. Gap junctions are clusters of intercellular channels that allow direct diffusion of ions and small molecules between adjacent cells (Bennett et al., 1991; Harris, 2001). Although the p.Arg42Cys variant has not been reported, a mutation changing the same amino acid (p.Arg42Pro) has been described to be associated with *Erythrokeratoderma variabilis* without deafness (Wilgoss et al., 1999; Richard et al., 2000). Both the proband and her normal hearing mother exhibited the p.Arg42Cys variant, but none had this skin disease. The fact that the unaffected mother also carried this variant indicates that this variant was not the main cause of hearing loss that segregated in the family, but it might have contributed to the phenotype as a modifier (Richards et al., 2015). Indeed, the proband presented a more severe hearing loss at a younger age (32 years) when compared to the other affected family members. There are many examples of the action of modifier genes in the disease expressivity for a number of diseases (i.e., pancreatitis, breast cancer, Gaucher disease, retinitis pigmentosa, hemochromatosis, hypertrophic

**Table 1** Mutations in *MYO6* reported to date.

dbSNP ID	Genomic	Nucleotide change	Position	Amino-acid	Inheritance	Phenotype	Ancestry	Onset	NMD	Reference
-	chr6:765273000→T	c.36_37insT	exon 2	p.Thr13Tyrfs*20	AR	HL, Vestibular dysf., facial dysmorphism. One individual with RP	Pakistani	Congenital	Yes	Ahmed et al., 2003
-	chr6:76527344:A>G	c.80A>G	exon 2	p.Asp27Gly	AR	NSHL	-	-	No	DVD
rs727504567	chr6:76538307:C>T	c.238C>T	exon 4	p.Arg80*	unknown	NSHL	-	-	Yes	DVD
-	chr6:76542558:G>A	c.392-1G>A	intron 5	-	AR	NSHL	Iranian	-	No	Yan et al., 2016
-	chr6:76550301:G>A	c.554-1G>A	intron 7	-	AD	NSHL, progressive	German	school age	No	Volk et al., 2013
rs772421255	chr6:76550332	c.584C>A	exon 8	p.Ala195Glu	AD	HL	Dutch	-	No	Zazo-Secco et al., 2017
-	chr6:76550362:G>A	c.614G>A	exon 8	p.Arg205Gln	-	NSHL, progressive	Japanese/Korean	?/First decade	No	Miyagawa et al., 2015; Kwon et al., 2014
rs557441143	chr6:76550361:C>T	c.613C>T	exon 8	p.Arg205*	AD	NSHL	Korean	-	Yes	Choi et al., 2013
-	chr6:76551013:A>G	c.734A>G	exon 9	p.Tyr245Cys	AR	NSHL	Chinese Hans	early-onset	No	Yang et al., 2013
rs121912560	chr6:76551016:A>G	c.737A>G	exon 9	p.His246Arg	-	NSHL, progressive and cardiomyopathy	-	5-54 years	No	Mohiddin et al., 2004
-	chr6:76554679:CCG delCCGC CAAAAGT>-	c.882_891 delCCGC AAAAGT	exon 10	p.Arg295Leufs*9	-	NSHL	German	1 year	Yes	Vona et al., 2014
-	chr6:76554694:G>T	c.897G>T	exon 10	p.Glu299Asp	Semi-dominant	NSHL, progressive/stable	Palestinian Arab	Congenital and late-onset	No	Brownstein et al., 2014
rs727503326	chr6:76554623:C>T	c.826C>T	exon 10	p.Arg276*	AR	NSHL	-	-	Yes	DVD
rs749752357	chr6:76554660:AC AA>-	c.863_866 delACAA	exon 10	p.Lys289 Argfs*17	AD	NSHL	-	-	Yes	Shearer et al., 2010
-	chr6:76558185:C>T	c.1015C>T	exon 11	p.Arg339Trp	AR	NSHL	Chinese Hans	early-onset	No	Yang et al., 2013
-	chr6:76564987:G>-	c.1210delG	exon 12	p.Gly404Gluufs*4	AD	NSHL	?/Dutch	-	Yes	Neveling et al., 2013; Zazo-Secco et al., 2017
rs121912557	chr6:76566915:G>A	c.1325G>A	exon 13	p.Cys442Tyr	AD	NSHL, progressive	Italian	childhood	No	Melchionda et al., 2001
-	chr6:76568688_76 568689insT	c.1452insT	exon 14	p.Asn485*	AD	NSHL	American	-	Yes	Yan et al., 2016
-	chr6:76570743_76570753delCAA GAACTCTA	c.1477_1487delCAA GAACTCTA	exon 13	p.Gln493Serfs*8	AR	NSHL	Nigerian	-	Yes	Yan et al., 2016
-	chr6:76570813:G>T	c.1546+1G>T	intron 15	-	AD	NSHL, progressive	?/Dutch	-	No	Neveling et al., 2013; Zazo-Secco et al., 2017
-	chr6:76572336:G>T	c.1570G>T	exon 16	p.Gly524*	AD	NSHL	-	-	Yes	DVD
rs773316449	chr6:76576249:A>G	c.1681A>G	exon 17	p.Arg561Gly	-	NSHL	-	congenital	No	Ji et al., 2014
-	chr6:76580394:C>T	c.1975C>T	exon 19	p.Arg659*	AD	NSHL	Japanese	-	Yes	Miyagawa et al., 2015

(Continued)

**Table 1** Continued.

dbSNP ID	Genomic	Nucleotide change	Position	Amino-acid	Inheritance	Phenotype	Ancestry	Onset	NMD	Reference
rs397517045	chr6:76589569:→G	c.2110_21 1insG	exon 21	p.Tyr705Leu <sup>fs</sup> *9	AD	NSHL	–	–	Yes	DVD
–	chr6:76591498:G>A	c.2379G>A	exon 23	p.Trp793*	AR	NSHL	–	–	Yes	DVD
–	chr6:76593963:T>G	c.2417-1758T>G	intron 23	–	AD	NSHL, progressive	Belgian	3rd decade	Partial*	Hilgert et al., 2008
–	chr6:76595777:C>T	c.2473C>T	exon 24	p.Arg825*	AD	NSHL, progressive	Korean	–	Yes	Kwon et al., 2014
rs121912561	chr6:76596598:C>T	c.2545C>T	exon 25	p.Arg849*	AD	NSHL	Danish	school age	Yes	Saanggaard et al., 2008
–	<b>chr6:76599832C&gt;A</b>	<b>c.2717C&gt;A</b>	<b>exon 26</b>	<b>p.Ser906*</b>	<b>AD</b>	<b>NSHL, progressive</b>	<b>Brazilian</b>	<b>19–60 years</b>	<b>Yes</b>	<b>This study</b>
–	chr6:76599867_7 6599868insA	c.2752insA	exon 26	p.Gln918A 1afs*941	AD	NSHL, progressive	Korean	5th decade	Yes	Kwon et al., 2014
–	chr6:76599892:T>A	c.2777T>A	exon 26	p.Leu926Gln	AR	NSHL	Arab-Palestinian	congenital	No	Brownstein et al., 2014
rs753233152	chr6:76599926:AA>--	c.2811_28 12delAA	exon 26	p.Arg939Ly s*fs19	AD	NSHL, progressive	Chinese	–	Yes	Cheng et al., 2014
–	chr6:76601021:C>T	c.2944C>T	exon 27	p.Gln982*	AD	NSHL, progressive	Chinese Hans	late-onset	Yes	Yang et al., 2013
–	chr6:76602271:C>T	c.2971C>T	exon 28	p.Arg991*	AD	NSHL, progressive	Korean	2nd decade	Yes	Kwon et al., 2014
rs727505015	chr6:76617352:TG	c.3207_3208 insATCCTA CATACTTA TTCCT	exon 31	p.Ala1070Ile <sup>fs</sup> *5	AD	NSHL	–	–	Yes	DVD
–	chr6:76618272:→G	c.3340_334 1insG	exon 32	p.Ala1114G1 yfs*5	AD?	NSHL	European	–	Yes	Schrauwen et al., 2012
–	chr6:76618327:delA	c.3395delA	exon 32	p.Lys1132Se rfs*12	AD	NSHL	Dutch	–	Yes	Zazo-Secco et al., 2017
–	chr6:76623855:G>A	c.3515G>A	exon 34	p.Arg1172His	AR	NSHL	–	–	No	DVD
–	chr6:76623950:C>T	c.3610C>T	exon 34	p.Arg1204Trp	AD	NSHL, progressive	Dutch	mid-life	No	Oonk et al., 2013
rs121912558	chr6:76623836:C>T	c.3496C>T	exon 34	p.Arg1166*	AR	NSHL	Pakistani	–	Yes	Ahmed et al., 2003
rs199699596	chr6:76624685:C>T	c.3814C>T	exon 35	p.Arg1272Trp	AD	NSHL	Japanese	–	No	Miyagawa et al., 2013

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; NSHL, nonsyndromic hearing loss; DVD, deafness variation database; RP, retinitis pigmentosa; HL, hearing loss.

All variant positions refer to NM\_004999, NMD information of Mutation Taster.

\*Information provided by Hilgert et al., 2008



**Table 2** Mutations in exon 2 of *GJB3* reported to date (NM\_024009). NMD is not predicted in any of these mutations.

db SNP ID	Genomic	Nucleotide change	Amino-acid	Inheritance	Phenotype	Ancestry	Onset	Reference
rs775072109	chr1:35250442:G>A	c.79G>A	p.Val27Met	AD	Hearing loss	Korean	–	Oh et al., 2013
–	chr1:35250487:C>T	c.124C>T	p.Arg42Cys	AD	<b>NSHL, progressive</b>	<b>Brazilian</b>	<b>19–60 years</b>	<b>This study</b>
–	chr1:35250506:AGA>–	c.143_145delAGA	p.Lys49del	AD	Deafness, non-syndromic	Brazilian	late onset	Alexandrinio et al., 2004
rs746219527	chr1:35250529:A>C	c.166A>C	p.Lys56Gln	AD	Hearing loss	German	–	Beck et al., 2015
rs777106179	chr1:35250557:ACG>–	c.194_196delACG	p.Asp66del	AD	Peripheral neuropathy & mild asymmetrical hearing impairment	–	–	Lopez-Bigas et al., 2001
rs765605645	chr1:35250665:G>A	c.302G>A	p.Arg101Gln	AD	Hearing loss	German	–	Beck et al., 2015
rs369979083	chr1:35250680:G>A	c.317G>A	p.Arg106His	AD	Hearing loss	German	–	Beck et al., 2015
rs74315320	chr1:35250784:A>G	c.421A>G	p.Ile141Val	AR	Deafness, non-syndromic	Chinese	early onset	Liu et al., 2000
rs770247378	chr1:35250784:ATT>–	c.421_423delATT	p.Ile141del	AR	Deafness, non-syndromic	Chinese	early onset	Liu et al., 2000
–	chr1:35250800:T>C	c.437T>C	p.Leu146Pro	AD	Deafness	Japanese	early onset	Miyagawa et al., 2013
–	chr1:35250822:G>T	c.459G>T	p.Trp153Cys	AD	Deafness	Japanese	late onset	Miyagawa et al., 2013
rs121908851	chr1:35250860:A>G	c.497A>G	p.Asp166Ser	AR (digenic with <i>GJB2</i> -235delC/+)	Deafness, non-syndromic	Chinese	Congenital	Liu et al., 2009
–	chr1:35250883:G>A	c.520G>A	p.Val174Met	AD	Deafness	Taiwanese	–	Yang et al., 2007
rs74315319	Chr 1:35250902:G>A	c.539C>A	p.Arg180*	AD	Progressive Deafness, non-syndromic	Chinese	20–30 y	Xia et al., 1998
rs74315318	Chr1:35250910:G>A	c.547G>A	p.Glu183Lys	AD	Progressive Deafness, non-syndromic	Chinese	~40 y	Xia et al., 1998
rs117385606	Chr1:35250943:G>A	c.580G>A	p.Ala194Thr	AR (digenic with <i>GJB2</i> -235delC/+ or 299delAT/+)	Deafness, non-syndromic	Chinese	Congenital	Liu et al., 2009
rs373815705	chr1:35251030:C>A	c.667C>A	p.Pro223Thr	AR (second mutation not detected)	Deafness, non-syndromic	Turkish	–	Uyguner et al., 2003
–	chr1:35251129:G>A	c.766G>A	p.Gly256Ser	?	Deafness	Chinese	–	Li et al., 2010

All variants were described in exon 2 (NM\_024009) and no NMD

and cardiomyopathy among others) as remarkably reviewed by Cooper et al. (2013). The variant p.Arg42Cys was considered as of uncertain significance.

Finally, the lack of mutations in known deafness genes in the remaining three families with autosomal dominant inheritance suggests that the majority of those genes remain to be revealed in the Brazilian population. Zazo-Seco et al. (2017) were unable to identify the causative variants in 66.5% (133/200) of HL patients after exome sequencing, and this rate was not so different from our study given the differences in the sample size.

## Conclusion

In conclusion, exome sequencing of four familial cases of autosomal dominant hearing loss revealed a novel nonsense *MYO6* mutation in one family. The exome sequencing of deaf families, after the pre-exclusion of known deafness genes, is one strategy that is advancing the determination of the contribution of each known deafness gene in a given population, advancing the establishment of new genotype–phenotype correlations and the discovery of novel deafness genes.

## Acknowledgements

The authors thank all family members for their participation in this hearing loss study, Prof. Dr. Ester Cerdeira Sabino (Department of the Infectious and Parasitic Diseases, Tropical Medicine Institute, University of São Paulo School of Medicine) and the Molecular Biology section of the DASA Group (Diagnósticos da América, Barueri – Brasil) for performing and funding the exome sequencing. We also thank Gleiciele Alice Vieira Silva for technical support. This work was financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Study design: JS-S, RFB, JO, KL; Data Collection & Analysis: JS-S, ACB, RJ-S, OA-N, LCS, PYN, LTG, KL. Manuscript preparation: JS-S, ACB, OA-N, JO, KL.

## Conflicts of interest statement

We declare no conflicts of interest.

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Received: 22 March 2017

Accepted: 16 August 2017