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REVIEW

Genetic etiology of non-syndromic hearing loss in Latin America

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Abstract
Latin America comprises all countries from South and Central America, in addition to Mexico. It is characterized by a complex mosaic of regions with heterogeneous genetic profiles regarding the geographical origin of the ancestors and proportions of admixture between the Native American, European and African components. In the first years following the findings of the role of the *GJB2/GJB6* genes in the etiology of hearing loss, most scientific investigations about the genetics of hearing loss in Latin America focused on assessing the frequencies of pathogenic variants in these genes. More recently, modern techniques allowed researchers in Latin America to make exciting contributions to the finding of new candidate genes, novel mechanisms of inheritance in previously known genes, and characterize a wide diversity of variants, many of them unique to Latin America. This review aimed to provide a general landscape of the genetic studies about non-syndromic hearing loss in Latin America and their main scientific contributions. It allows the conclusion that, although there are similar contributions of some genes, such as *GJB2/GJB6*, when compared to European and North American countries, Latin American populations revealed some peculiarities that indicate the need for tailored strategies of screening and diagnosis to specific geographic regions.

Introduction

The origin of present Latin America populations

The Americas were the last continent to be colonized by humans, about 30,000 and 70,000 years later than the other continents. Most studies pointed out that the pioneer humans in America arrived from northeastern Asia, now Siberia, at least ~ 14,500 years before present (BP) (Raghavan et al. 2015; Llamas et al. 2016; Potter et al. 2017; Posth et al. 2018). There were also subsequent waves of migration from Siberia that left genetic signatures on some populations (Reich et al. 2012). Native American populations remained isolated from the populations in the other continents for nearly 10,000 years until they contacted the Europeans 500 years ago (Salzano and Sanz 2014). The contact with Europeans led to reductions in population size and severe bottlenecks. These facts explain why there are specific alleles or allele frequencies among the Amerindians (Native Americans) and significant genetic differentiation. Amerindian ancestry is especially prevalent in Mexico, Guatemala, Peru, Ecuador, and the Caribbean area and varies among Latin American countries (Salzano and Sanz 2014; Rodrigues-Soares et al. 2020). Genomic datasets have brought essential knowledge regarding the history and demography of these native populations, dividing autochthonous populations into significant groups such as Mesoamericans, Andeans, Amazonians, and Eskimos (Reich et al. 2012; Hünemeier et al. 2012).

Geographically, what we call Latin America presently comprises all countries from South and Central America and Mexico. Estimates indicate that, when the Europeans started their migration to the American continent in 1492, about 45 million Amerindians were in Latin America (Salzano and Bortolini 2002). Genetically different Amerindian populations contacted different European populations, with different relationships that provided more or less interbreeding (Tamm et al. 2007). Regarding the European component, the first and more representative colonizers were from Spain, in almost every region except Brazil, which the Portuguese colonized. The predominant Portuguese presence in its first

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three centuries of colonization explains why Brazil is the only Portuguese-speaking country in Latin America.

After the Europeans, the African slaves came to America from many different parts and ethnicities from Africa (Klein 1999). As a result, different parts of the American continent received different amounts of African genetic contributions from different ethnicities (Gouveia et al. 2020). Furthermore, it was frequent that one geographical region received Africans from different regions, resulting in a complex mosaic of regions with heterogeneous genetic profiles, regarding the geographical origin of the ancestors and proportions of admixture between the Native American, European and African components (Gouveia et al. 2020). In addition, East Indian, Chinese, Japanese, Javanese, and other Asian populations also migrated later to Latin America, increasing the diversity of our genetic backgrounds (Salzano and Sans 2014).

Brazil is the largest country from Latin America, with one of the most genetically heterogeneous populations in the world, due to its complex and comprehensive admixture between Amerindians, Africans, and Europeans from the past 500 years, and more recent admixture with Middle Eastern and East Asian migrants (Carvalho-Silva et al. 2001). Slaves came in waves of forced migration and with different origins from Africa, ranging from the Guinea coast to Mozambique, between the XVI and the XIX centuries (Klein 1999; Gouveia et al. 2020). Later on, starting in the nineteenth century, Germans, Japanese, Poles, Lebanese, Syrians, French, Ukrainians, Lithuanians, Jews, Russians, and many others migrated to Brazil (IBGE 2000; Ongaro et al. 2019).

Brazil is a country of continental size with nearly 210 million people and has a history of massive interethnic admixture. In a simplified model, the Brazilian population can be described as three-hybrid composed of Amerindians, Europeans, and Africans. However, the proportion of the contribution of each parental population and the frequency of interethnic admixture varied between different regions of Brazil, as a consequence of historical patterns, migration, and economic dynamics, making each Brazilian city or region unique in terms of ancestry composition and allele frequencies. Consequently, this mosaic of ethnicities profoundly impacts the dynamics and distribution of mutations that lead to genetic diseases.

Overview of hearing loss In Latin America

Latin America is a region of contrasts, with a significant part of its diverse population lacking health care assistance and facing absolute poverty (Madriz 2000), but interspersed with nuclei of intense development. Moreover, it comprises a vast area composed of distinct environments regarding geological, climate, altitude, and ecological aspects (Salzano and Sans 2014). Despite the advances in the genomic and medical fields, the lack of accessibility to resources and health leadership makes those advances intangible for a significant fraction of the population. However, modern research centers arose, mainly in the Universities, giving rise to high-quality medical and genetic services, allowing genetic counseling and genetic tests, including those based on massive parallel sequencing. Nevertheless, these services are still restricted in numbers and concentrated in few urban centers. Unfortunately, they are not accessible to everyone who needs them, but they have allowed extracting interesting information about the genetics of hearing loss.

Despite the large number of children being born every year in Latin American countries, several barriers delayed the implementation of UNHS (universal newborn hearing screening) in all countries, such as limited funding, inadequate support services, and shortage of qualified personnel. Although some countries have taken necessary steps towards introducing NHS, evidence from the literature suggests that hearing loss has not received high priority in many parts of Latin America (Gerner de Garcia et al. 2011). Argentina and Brazil have been addressing NHS efforts since the 1990s, and, in Argentina, legislation from 2001 defines that all newborns have the right to be screened for hearing loss and to receive appropriate diagnostic evaluation and treatment. Brazil has the largest and the oldest NHS programs in Latin America, with many screening sites in different states. However, the law that determines that Universal Neonatal Hearing Screening is mandatory in all children was approved only in 2010.

In an interesting survey on the topic (Gerner de Garcia et al. 2011), the authors concluded that there is great variation in the strategies to identify infants with hearing loss within Latin America, ranging from efforts restricted to a single hospital or region of a country, to some programs implemented at the national level. It seems that in countries with higher incomes per capita, such as Mexico and Brazil, it is more likely that the resources needed to implement UNHS are regularly provided; on the other hand, in countries with limited funding, although there are growing efforts to implement screening programs, they are restricted to few hospitals or regions. Likewise, comprehensive and systematic surveys about frequency and the different causes of HL are scarce as the access to molecular diagnosis.

Given the heterogeneity of study coverage and strategies of NHS in the different regions and countries in Latin America, it is far from being an easy task to obtain a reliable picture of the prevalence of hearing loss cases and their causes.

Few published studies are reporting the results of Universal Newborn Hearing Screening (UNHS) in Brazil. One of the first, the study of Chapchap and Serge (2000) was performed in a private hospital in the city of São Paulo and disclosed a prevalence of 2.4/1000 affected newborns. In

the sample of Bevilacqua et al. (2010), studied in a public hospital in the city of Bauru, in the state of São Paulo, the prevalence of sensorineural congenital hearing loss was estimated in 0.96/1000. According to the Universal Neonatal Hearing Screening program leadership of the city of São Paulo (Manzoni et al. 2016), the prevalence of permanent hearing loss in the screened population was estimated to be 1–2/1000 newborns. Also, in the state of São Paulo (in Jundiaí), the prevalence was estimated to be 1.9/1000 (Pereira et al. 2014), and in 2/1000 in the city of Ribeirão Preto (Anastasio et al. 2021). In other Brazilian states, in Santa Catarina, a figure of 3.2/1000 was obtained (Mattos et al. 2009). In recently evaluated samples from the Federal District and the Belo Horizonte city (State of Minas Gerais), estimates of 3/1000 (Marinho et al.2020) and near 4/1000 (Barboza et al. 2013) were found.

Martínez-Cruz et al. (2020), in Mexico, through the investigation of an extensive series of 14.000 neonates, provided an estimate of the prevalence of hearing loss of 2.2/1000.

Although available data are scattered, overall, it seems that in developed Latin American cities where UNHS could be efficiently implemented, the estimated prevalence of hearing loss is similar to the values obtained in developed countries, 1–2/1000, with some samples providing evidence that these values may reach 3–4/1000. In parallel to the implementation of UNHS, cochlear implantation is slowly surpassing the challenges and reaching a growing number of children with profound hearing loss in Latin America. A study about the cost-effectiveness of cochlear implantation in Latin America shows that the number of implants per year is heterogeneous among countries. However, some of them perform appreciable numbers of implants annually, such as 1200 and 500, in Brazil and Colombia, respectively. However, these numbers represent much less than 50% of the potential cases that would benefit from the procedure (Emmett et al. 2016).

The relative contribution of environmental and genetic factors to the etiology of hearing loss is highly correlated to the level of socio-economic development, ethnicity, and demographic region. Although much progress related to prenatal and neonatal health care and immunization programs has been made in recent years, complications in these critical periods, congenital or neonatal infections, and their management, are still significant causes of hearing loss, especially in developing countries of Latin America.

There are few comprehensive epidemiological and systematic studies about the proportion of syndromic features among patients with hearing loss. Ideally, centers with standard guidelines for diagnosing both environmental and genetic causes should be the ones to investigate the proportion of syndromic and non-syndromic hearing loss cases. However, whenever the environmental factors were the focus of the few studies available, the role of genetic factors was misinterpreted or was not profoundly investigated. Genetic Services performed a few reliable studies. In many publications, the syndromic cases were excluded from the investigation to maximize the molecular diagnostic rate. Thus, different types of bias, for example, in the inclusion criteria, are likely to be influencing the numbers. For instance, Tamayo et al. (2009) analyzed data from 731 deaf children attending 8 schools for the deaf in Bogotá and identified 322 (44%) with no malformations or mental retardation. The ophthalmologic evaluation suggested congenital rubella in ~ 20% (in the non-syndromic group) and other anomalies in 5.8%.

In Brazil, syndromic features were identified in 12.5% of all hearing loss cases studied from two rural communities from Paraiba State by Melo et al. (2014). In contrast, in São Paulo, Batissoco et al. (2021, this issue) found syndromic features in 19% of the patients referred to the Genetic Service of the Otorhinolaryngology Department. Faistauer et al. (2021, in the State of Rio Grande do Sul) found 33% of syndromic cases among the subjects with a likely genetic cause for hearing loss.

In addition to genetic syndromes, some environmental factors, such as congenital infections, are likely the causes of syndromic features.

There are few studies about the contribution of environmental and genetic factors to the etiology of prelingual hearing loss in Latin American countries published in indexed peer-reviewed journals. Nevertheless, some studies provided some perspective into the field. In Brazil, congenital infections were reported among prelingual hearing loss cases, with prevalence ranging from 6 to 29% (Faistauer et al. 2021; Botelho et al. 2010; Anastacio et al. 2021). Other important environmental risk factors were prolonged admission to neonatal ICU, mechanical ventilation, ototoxic use, exchange transfusion for neonatal jaundice, and hyperbilirubinemia (Pereira et al. 2014; Botelho et al. 2010; Anastacio et al. 2021; Faistauer et al. 2021). Ramos et al. (2013) studied 100 unrelated subjects with bilateral severe to profound SNHL submitted to the cochlear implant and encountered 8% of congenital infections, postnatal infection in 4%, prematurity/neonatal ICU stay in 3%, and 19% of DFNB1-related hearing loss.

In the first years following the remarkable findings of the role of the *GJB2*/*GJB6* genes in the etiology of hearing loss (Zelante et al.1997; Denoyelle et al. 1997; Del Castillo et al. 2005), the majority of scientific studies regarding the genetics of hearing loss in Latin America aimed to evaluate the frequencies of pathogenic variants in the *GJB2/GJB6* genes, to develop affordable genetic tests that could bring an immediate and relevant contribution for diagnosis, genetic counseling, and prognosis. Unfortunately, while Sanger sequencing proved worthier, especially for populations with

diverse ancestries other than European, in many studies, only the c.35delG screening was affordable.

Later, specific investigations were performed on the frequency of variants in known genes, such as *OTOF* and *SLC26A4,* based mainly on conventional Sanger sequencing techniques. More recently, the application of massive parallel sequencing to diagnosis and research allowed the scientists in Latin America to make exciting contributions to the finding of new candidates genes (Lezirovitz et al. 2020; Salazar-Silva et al. 2021) to highlight novel mechanisms of inheritance in previously known genes (Dantas et al. 2018; Dias et al. 2019), and to characterize a wide diversity of variants, many of them were never described in other continents (Lezirovitz et al. 2008,2012; Batissoco et al. 2009a, b; Romanos et al. 2009; Uehara et al. 2015; Bademci et al. 2016; Nonose et al. 2018; Sampaio-Silva et al. 2018; Dantas et al. 2018; Dias et al. 2019). Although NGS is nowadays widely employed in developed countries in the molecular diagnosis for HL, this is far from becoming a reality in Latin America since, in the majority of regions, even the *GJB2/GJB6* screening is not available to every patient or, even worse, many patients with hearing loss never receive proper hearing rehabilitation or can reach a Genetic Service. Thus, molecular diagnosis for HL is not part of the public health care system in many Latin American countries.

Thus, the genetic heterogeneity of non-syndromic hearing loss (NSHL) makes its molecular diagnosis a real challenge, mainly for those underdeveloped countries that form Latin America. Since there are hundreds of genes responsible for this phenotype, their identification is troublesome and expensive. Furthermore, the contribution of each gene/genetic cause may vary significantly in different Latin American populations. Thus, determining the most frequently altered genes and variants in Latin America could help prioritize the genes and variants to be screened, providing a rationale for developing cheaper and effective screening strategies with direct applications to genetic counseling.

This review aimed to provide a general landscape of the genetics of non-syndromic hearing loss studies in Latin America and highlight their main scientific contributions.

Methods

Search strategy

The search was done in the following databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://apps.webofknowledge.com/) and LILACS (https://lilacs.bvsalud.org/). The keywords were "country or region from Latin America" and "genetic" + "hearing loss/deafness", or "*GJB2*", "*SLC26A4*", "Pendred", "nonsyndromic/non-syndromic hearing loss/deafness". Publications cited in the Hereditary Hearing Loss Homepage were also analyzed, and papers reporting Latin American pedigrees were selected.

The countries that comprised Latin America and were used as search terms were: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Guiana, French Guiana, Paraguay, Peru, Suriname, Uruguay, Venezuela, Mexico, Cuba, the Dominican Republic, Haiti, Honduras, Saint Barthelemy, Saint Martin, Martinique, and Guadeloupe.

Some studies came up as the result of searches for more than one country, such as Rodríguez-Ballesteros et al. (2008) and Bademci et al. (2016). The following countries/region terms did not yield publications or did not have any publication within the inclusion criteria: Latin America, Uruguay, Paraguay, Bolivia, French Guyana, Guyana, Suriname, El Salvador, Haiti, The Dominican Republic, Guadeloupe, Belize, Honduras, Martinique, and Saint Barthelemy.

Inclusion and exclusion criteria

Only articles in English were included (articles in Portuguese or Spanish were not). Reports of single cases about well-known genotype–phenotype correlations were not included. This systematic review focused on the studies on the molecular-genetic diagnosis of non-syndromic hearing loss. Pendred syndrome was exceptionally included since it belongs to one extreme of the phenotypic spectrum of variants associated with *SLC26A4*; the other extreme is DFNB4, a form of autosomal recessive non-syndromic hearing loss. Likewise, some variants in *GJB2* that can be associated with both non-syndromic HL and syndromic HL with skin/nail abnormalities exhibiting intrafamilial expression variability were also included.

The exclusion criteria were: irrelevant title or abstract; not related to Latin American countries in the search; not related to hearing loss or genetic; not about a disease (non-syndromic HL of genetic origin) included.

The number of publications per country and among the different topics (*GJB2/GJB6*, mitochondrial, *SLC26A4*, *OTOF*, and other genes) before and after the exclusion criteria were applied are presented in Supplementary Fig. S1. In total, 828 papers were retrieved, and 88 publications were included, 60% from Brazil. In addition, a manuscript in this same issue was included (Batissoco et al. 2021). The main topic was *GJB2/GJB6*, representing 53.4% of the papers. Some papers included more than one topic, and they were classified according to their most important findings.

Data extraction

The first author (KL) did the searching process on two different occasions, separated by a couple of months. Both authors (KL and RCMN) reviewed all the titles independently. First, differences in articles to be included were discussed to find a consensus uniform criterion. Next, the full texts were analyzed to access relevance. Bouzaher et al. (2020), a systematic review of *GJB2* variants in the Latino population, was not included, although it appeared in the majority of the searches, because it contains no original research data.

Quality assessment

Both authors evaluated the risk of bias assessment on all articles, and potential factors of bias or limitations were indicated in Table 1 (italic), in Supplementary Table S1, and the results section. We were aware that the selection criteria of individuals in each study varied. In most cases, they were biased to maximize molecular diagnosis because of the low budget, instead of providing a representative sampling of the populations in which the subjects were ascertained. The possible bias factors, which were taken into consideration for interpretation of the results were: selection of samples to maximize molecular diagnosis such as familial cases or most frequently associated phenotypes, motivation of the study, the inclusion of related probands, not performing mutational analysis of the whole genes, the inclusion of benign and pathogenic variants in the results (in these cases, the raw numbers were assessed and reanalyzed). When many papers of the same study group were published within few years and with quite similar data, without proper reference, the paper with the largest cohort or with the less biased sample selection was chosen for frequency calculations and to construct the pie charts. The frequencies were compared without any statistical analysis since the inclusion criteria, cohort selection and size, and molecular strategies were highly heterogeneous.

A *GJB2/GJB6* test was considered complete if c.35delG (NM_004004.6:c.35del) was screened, if sequencing was performed in at least the heterozygous cases and if the most frequent *GJB6* deletions were tested (del(*GJB6*-D13S1830) and del(*GJB6-D13S1854*). Ideally, the *GJB2* complete coding region should be analyzed by sequencing, the *GJB6* deletions should be tested, and for those who remained monoallelic, the c.–23 + 1G > A should also be screened. Nonetheless, all publications were included in Table 1 (and Supplementary Table S3), and the ones without complete tests were considered, with risk of molecular bias, but this was indicated.

The knowledge about the pathogenicity status of variants might have changed in time, with the increase of genotype–phenotype correlation studies and functional studies. Thus, in this review, we summarized data in tables following the guidelines of ACMG (Richards et al. 2015; Oza et al. 2018) and using the present revised classification of pathogenicity of each variant consulting the Deafness Variation Database (https://deafnessvariationdatabase.org/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), which may not always coincide with the classification of the variant at the time the original paper was published.

Results and discussion

To provide an organized set of data and make it clear to readers, our results were divided into several topics regarding the different genes or mechanisms of transmission of hearing loss.

The contribution of *GJB2/GJB6* variants to the etiology of hearing loss in different Latin American populations

The c.35delG (NM_004004.6:c.35del; NP_003995.2: p.Gly12ValfsTer2) is the most frequent pathogenic variant found in *GJB2* across many different populations distributed worldwide. It probably has a European origin; thus, its frequency in multiethnic countries with admixed populations might reflect the contribution of European ancestry to these populations. Rothrock et al. (2003) genotyped SNPs from chromosomes bearing the c.35delG variant and close microsatellite markers in patients with hearing loss from different countries such as Italy, Brazil, and North America. Their study provided evidence that the c.35delG variant arose in European and Middle Eastern populations through a single mutational founder event. Numerous studies revealed that the carrier frequency varies among different hearing populations, being as high as 1 in 31 in Caucasians from the Mediterranean (Estivill et al. 1998b; Gasparini et al. 2000). The low frequency of c.35delG among non-European descendants, such as the Japanese or the African populations, suggested that c.35delG was not a recurrent mutation, but its present distribution is the consequence of a founder event followed by migrations (Gasparini et al. 2000).

In 2000, Sartorato et al. screened the c.35delG variant in 620 randomly selected neonates from the region of Campinas, São Paulo State—Brazil, to determine its frequency in the general population. Carrier rate was estimated as 0.97% (frequency of heterozygotes), or 1 in 103, and the allelic frequency of this variant (35delG chromosomal rate) was

Table 1 Summary of the studies about the contribution of the *GJB2/GJB6* genes to HL in Latin America

Country (Region)	City—State	[Ref]	Reference	*GJB2/GJB6* DR	c.35delG/mutated alleles*	c.35delG/all chrs	Most common variants (-c.35delG)	Inclusion criteria	Methods
Brazil (S)	PR, SC	1	Batissoco et al. (2021)	14.3% (3/21)	100% (6/6)	13.6% (6/44)	–	Bi,Uni/PR, PP/all/SN, MX, CT/S, NS, MF/I/39/9	*GJB6*dels, *GJB2*seq, nc*GJB2*
Brazil (S)	Porto Alegre—RS	2	Da Motta et al. (2012)	8.1% (3/37)	100% (10/10)	11.1% (8/72)	–	-/PR/P/SN/E/NS/-/-	*GJB2seq*
Brazil (S)	Porto Alegre—RS	3	Faistauer et al. (2021)	9.9% (13/131)	90% (27/30)	10.3% (27/262)	DEL(GJB6-D13S1830)	Bi/PR/-/SN, Mx/E/NS/19.3/-	*GJB6dels, GJB2seq*
Brazil (SE)	Campinas—SP	4	Oliveira et al. (2002)	11.3% (7/62)	84% (16/19)	12.9% (16/124)	p.(Val95Met) p.(Val37Ile) p.(Glu120del)	*-/PR, PP/-/SN/E/NS/16/-*	*GJB2seq*[#]
Brazil (SE)	Campinas—SP	5	Bernardes et al. (2006)	12% (4/32)	100% (14/14)	21.9% (14/64)	–	*Bi/PR/P/SN, Mx/-/NS/-/-*	*c.35delG*
Brazil (SE)	Campinas—SP	6	Oliveira et al. (2007b)	7.1% (46/645)	75% (84/112)	6.5% (84/1290)	p.(Met34Thr)	-/-/all/SN/E/NS/-/-	*c.35delG, GJB6dels, GJB2seq*
Brazil (SE)	Campinas—SP	7	Christiani et al. (2007)	22.5% (11/49)	82.8% (24/26)	24.5% (24/98)	DEL(GJB6-D13S1830)	-/-/S, P/SN/E/NS/-/-	*GJB6dels, GJB2seq*
Brazil (SE)	Campinas—SP	8	da Silva-Costa et al. (2011)	7.5% (45/600)	87% (94/108)	7.8% (94/1200)	c.-23 + 1G > A	*-/-/M, P/SN/E/NS/-/-*	*GJB6 dels, GJB2 seq, ncGJB2, MLPA of GJB2, GJB3, and GJB6*
Brazil (SE)	Campinas—SP	9	Martins et al. (2013)	21.3% (60/282)	72% (80/110)	14.2% (80/564)	p.(Val95Met) p.(Val37Ile) p.(Met34Thr)	*-/-/-/SN/E/NS/-/-*	*Optimization of the TaqMan® OpenArray™ Genotyping Platform for NSSNHL—31 variants in GJB2, GJB6, CRYL1, TMC1, SLC26A4, miR-96, and OTOF, MTRNR1 MT-TS1*
Brazil (SE)	Campinas—SP	10	Ramos et al. (2013)	18% (18/100)	78.6% (22/42)	16.5% (33/200)	DEL(GJB6-D13S1830) p.(Met34Thr)	Bi/PR, PP/S, P/SN/E/NS/40/2	*c.35delG, GJB6dels, GJB2seq*
Brazil (SE)	Campinas—SP	11	Svidnicki et al. (2015)	17.8% (32/180)	69% (49/71)	13.6% (49/360)	p.(Met34Thr) DEL(GJB6-D13S1830) p.(Trp24Ter) p.(Val37Ile)	*Bi/PR, PP/all/SN/E/NS/-/-*	*MassARRAY iPLEX® technology*
Brazil (SE)	São José do Rio Preto—SP	12	Belintani Piatto et al. (2004)	21.2% (7/33)	87.5% (14/16)	21.2% (14/66)	p.(Val37Ile)	Bi/PR/all/SN/E/NS/-/-	*c.35delG, GJB6dels, GJB2seq*

Table 1 (continued)

Country (Region)	City—State	[Ref]	Reference	*GJB2/GJB6* DR	c.35delG/mutated alleles*	c.35delG/all chrs	Most common variants (-c.35delG)	Inclusion criteria	Methods
Brazil (SE)	Marília—SP	13	Esteves et al. (2014)	1.0% (1/101)	88.9% (8/9)	4.0% (8/202)	DEL(GJB6-D13S1830)	*Bi/PR, PP/all/SN/E/NS/-/-*	*c.35delG, GJB6dels*
Brazil (SE)	Botucatu—SP	14	Moreira et al. (2015)	1.7% (1/58)	—	3.5% (4/116)	—–	-/PR, PP/all/SN/-/NS/-/-	*c.35delG*
Brazil (SE)	Ribeirão Preto—SP	15	Carvalho et al. (2018)	10.2% (9/88)	73.9% (17/23)	9.7% (17/176)	DEL(GJB6-D13S1854)	-/PR/all/SN/-/NS/28.4/13.6	*GJB6dels, GJB2seq*
Brazil (SE)	São Paulo -SP	16	Batissoco et al. (2009a, b)	12% (36/301)	68% (59/87)	9.8% (59/602)	p.(Met34Thr) DEL(GJB6-D13S1830)	-/PR, PP/all/-/E/NS, S/37.6/5.7	c.35delG, 167delT, *GJB6* dels, *GJB2* seq, GJB2 nc, MLPA, SSCP
Brazil (SE)	SP, RJ, ES, MG	1	Batissoco et al. (2021)	12.9% (55/425)	69.7% (85/122)	10% (85/852)	p.(Val37Ile) p.(Trp24Ter) DEL(GJB6-D13S1830) DEL(GJB6-D13S1854)	Bi,Uni/PR, PP/all/SN, MX, CT/S, NS, MF/I/39/9	*GJB6* dels, *GJB2* seq, nc*GJB2*
Brazil (SE)	Vitória—ES	17	Cordeiro-Silva et al. (2010, 2011)	6.5% (5/77)	85.7% (12/14)	7.8% (12/154)	DEL(GJB6-D13S1830)	Bi/-/M, P/SN/E/NS/-/-	c35delG, c.167delT/c.235delC/p.(Trp24Ter) tests, *GJB6*dels
Brazil (SE)	MG	18	Schüffner et al. (2020)	0% (0/53)	100% (9/9)	8.49% (9/106)	–	-/PR, PP/all/SN/E/NS/12.1/9.6	*c.35delG, GJB6dels*
Brazil (SE)	RJ	19	Felix et al. (2014)	4.0% (4/100)	64.0% (16/25)	8% (16/200)	p.(Met34Thr) p.(Ser199Glnfs-Ter9)	*-/PR, PP/S, P/SN, MX/E/NS/37.0/5.0, only c.35delG negative or heterozygous included*	*GJB2 seq*
Brazil (MW)	GO, MT, MS	1	Batissoco et al. (2021)	15.2% (3/17)**	20% (1/5)	3.2% (1/31)	–	Bi,Uni/PR, PP/all/SN, MX, CT/S, NS, MF/I/39/9	*GJB6*dels, *GJB2*seq, nc*GJB2*
Brazil (N)	TO, AM, PA, AC, RO	1	Batissoco et al. (2021)	7.7% (2/26)	100% (4/4)	7.7% (4/52)	–	Bi,Uni/PR, PP/all/SN, MX, CT/S, NS, MF/I/39/9	*GJB6*dels, *GJB2*seq, nc*GJB2*
Brazil (NE)	Monte Santo—BA	20	Manzoli et a. (2013)	37.0% (30/81)	80% (40/50)	24.7% (40/162)	p.(Arg75Gln)[#]	Bi/PR, PP/all/SN/I/NS/95/52.2	c.35delG, GJB6 *dels, GJB2* seq
Brazil (NE)	BA, PI, AL, MA, CE, PE, PB, SE	1	Batissoco et al. (2021)	15.2% (7/46)	68.8% (11/16)	12% (11/92)	p.(Val37Ile)	Bi,Uni/PR, PP/all/SN, MX, CT/S, NS, MF/I/39%/9%	*GJB6*dels, *GJB2*seq, nc*GJB2*

Table 1 (continued)

Country (Region)	City—State	[Ref]	Reference	*GJB2/GJB6* DR	c.35delG/mutated alleles*	c.35delG/all chrs	Most common variants (-c.35delG)	Inclusion criteria	Methods
Brazil (NE)	Queimadas—PB	21	Melo et al. (2014)	9.2% (7/76)	100% (21/21)	13.8% (21/152)	—–	-/PR, PP/all/-/I/NS/-/28.4	c.35delG, 167delT, *GJB6* dels, *GJB2* seq, *GJB2* nc, MLPA, SSCP
Brazil (NE)	Gado Bravo—PB			9.1% (4/44)	54.5% (6/11)	6.8% (6/88)	DEL(GJB6-D13S1854)	-/PR, PP/all/-/I/NS/-/21.8	
Brazil (N)	Belém—PA	22	Castro et al. (2013)	1.3% (1/77)	80% (8/10)	5.2% (8/154)	p.(Met34Thr) p.(Val95Met) p.(Trp172Ter)	-/PR/S, P/SN/E/NS/-/-	*GJB2 seq*
Ecuador	12 provinces	23	Paz-y-Miño et al. (2014)	50% (13/26)	3.4% (1/29)	1.9% (1/52)	p.(Gln7Ter) p.(Arg32Leu) p.(Thr77Arg) p.(Arg143Trp) p.(Ser199Phe) p.(Asn206Ser)	-/PR/all/SN, MX/-/NS/-/-	*GJB6dels, GJB2seq*
Nicaragua	Jinotega	24	Saunders et al. (2007)	0% (0/86)	100% (1/1)	0.6% (1/172)	–	-/PR, PP/all/-/E/NS/24–33/-	*GJB2seq*
Guatemala	different regions	25	Carranza et al. (2016)	8.3% (11/133)	0% (0/24)	0% (0/266)	p.(Trp44Ter) p.(Arg32Cys) p.(Val84Leu) p.(Glu147Lys)	-/PR/M,P/-/-/NS/17.3/-	*GJB2 seq*
Colombia	Bogotá	26	Tamayo et al. (2009)	31.2% (35/112)	46.3% (38/82)	17% (38/224)	p.Ser199Phe	Bi, Uni/PR, PP/all/SN, MX/E/NS/59.8/-	*GJB2seq*
Colombia	Providencia Island	27	Lattig et al. (2008)	47% (8/17)	100% (16/16)	47% (16/34)	–	Bi/PR/M, P/SN/-/NS, S/76.4/-	*GJB2seq*
Chile	–	28	Cifuentes et al. (2013)	5.3% (6/113)	100% (28/28)	12.4% (28/226)	–	Bi, Uni/PR, PP/M, P/SN/E/NS/20.4/-	*c.35delG, GJB2seq*
Peru	Lima	29	Figueroa-Ildefonso et al. (2019)	32.3% (43/133)	17.2% (16/93)	6% (16/266)	p.(Gly12Val) p.(Ile20Thr) p.(Arg32Ser) p.(Val95Met) p.(Arg143Trp) c.645del	-/PR/M, P/-/E/NS/47.4/—all previously negative for *GJB6*-dels probably biased towards familial cases	*GJB2*seq
Venezuela	Caracas	30	Angeli et al. (2000)	4.8% (2/42)	100% (5/5)	7.8% (5/64)	–	-/PR/M, P/SN/I/NS, S, MF/38.1/-	*c.35delG, SSCP*
Venezuela	Caracas	31	Utrera et al. (2007)	7.5% (3/40)	92.8% (13/14)	16.2% (13/80)	DEL(GJB6-D13S1830)	-/PR/M, P/SN/E/NS/82.5/-	*c.35delG, GJB6 dels, GJB2 seq*

Table 1 (continued)

Country (Region)	City—State	[Ref]	Reference	*GJB2/GJB6* DR	c.35delG/mutated alleles*	c.35delG/all chrs	Most common variants (-c.35delG)	Inclusion criteria	Methods
Argentina	Buenos Aires	32	Gravina et al. (2010)	34% (32/94)	61.5% (40/65)	21.3% (40/188)	p.(Arg143Trp) DEL(GJB6-D13S1830)	Bi/PR/M, P/SN/E/NS/10.6/-	c.35delG, *GJB6*dels, *GJB2*seq, nc*GJB2*
Argentina	Buenos Aires	33	Buonfiglio et al. (2020)**	16.2% (97/600)	48.5% (111/229)	9.2% (111/1200)	p.(Met34Thr) p.(Val37Ile) c.167delT p.(Arg143Trp) DEL(GJB6-D13S1830)	-/PR, PP/M, P/SN/E/NS/20/-	*GJB6* dels, *GJB2* seq, nc*GJB2*
Mexico	Mexico City	34	Arenas-Sordo et al. (2012)	11.8% (9/76)	60% (12/20)	7.9% (12/152)	c.167delT p.(Val84Leu)	-/PR/M, P/SN/E/NS/6.6/-	GJB6 dels, *GJB2* seq, nc*GJB2*
Mexico	Various Regions	35	Loeza-Becerra et al. (2014)	17.1% (24/140)	34.6% (36/104)	12.8% (36/280)	p.(Val84Met) p.(Phe31Ile) p.(Arg32Ser)	Bi/PR/M, P/SN/E/NS/8.6/2.1	*GJB6* dels, *GJB2* seq
Mexico	Mexico City	36	Hernández-Juárez et al. (2014)	3.8% (3/78)	46.7% (7/15)	4.5% (7/156)	p.(Gly12Val) c.645del	-/PR/S, P/SN/E/NS/-/-	*GJB6* dels, *GJB2* seq, nc*GJB2*
Mexico	Mexico City	37	Martínez-Saucedo et al. (2015)	2 cases of two likely pathogenic variants in *cis* and a third in *trans*: 1 sporadic case: p.(Ser19Arg) + p.(Arg32Ser)/p.(Glu47Ter); 2 siblings: p.(Phe31Ile) + p.(Val84Met)/p.Trp44Ter				-/PR/-/sn/E/NS/2 unrelated families + 100 controls	*GJB2 seq*

Studies are ordered, and those conducted in the same region are closer to each other. Biased studies concerning inclusion criteria or incomplete *GJB2/GJB6* test are italicized

Only biallelic recessive or monoallelic dominant cases were considered in the estimates of *GJB2/GJB6* contribution

Inclusion criteria: Laterality (Bi = bilateral, Uni = Unilateral)/ Onset (PR = prelingual, PP = postlingual)/ Severity (Moderate = M/Severe = S/Profound = P)/ Type (SN = Sensorineural, MX = Mixed)/ Environmental causes (I = included; E = excluded)/ SS = Syndromic features included, NS = Non-syndromic, MF = Malformation/ Familial history (%)/ Consanguinity (%). *GJB2*-nc = *GJB2* noncoding variants; *GJB6*-dels = *GJB6* deletions # published before the publications describing *GJB6* deletions or pathogenic variants in the exon 1-intron 1 of *GJB2*. South region States: Paraná (PR), Santa Catarina (SC), Rio Grande do Sul (RS); Southeast region States: São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Espirito Santo (ES); Northeast region States: Ceará (CE), Bahia (BA), Maranhão (MA), Pernambuco (PE), Alagoas (AL), Paraíba (PB), Sergipe (SE), Piauí (PI); North region States: Pará (PA), Tocantins (TO), Amazonas (AM), Acre (AC), Rondônia (RO); Midwest region States: Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS)

*Monoallelic cases were included in calculating the allele frequencies

**Includes the 476 in Dalamón et al (2013), that include 252 NSSNHL w/o environmental factors described in Dalamón et al. (2010), from which 44 prelingual and 2 postlingual were described in Dalamón et al. (2005)

#Dominant variant usually associated with HL and Keratoderma

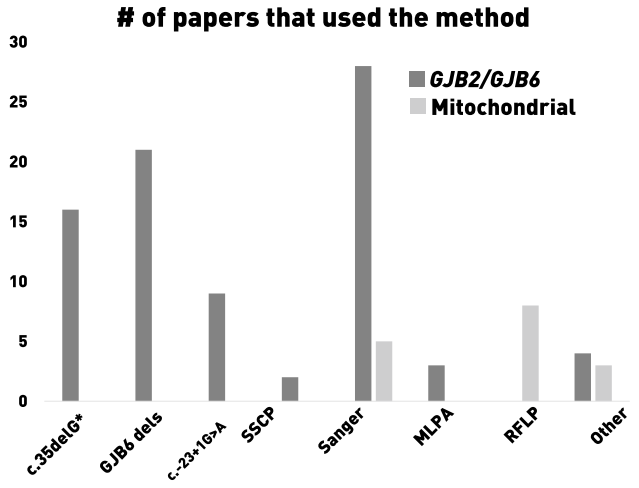


Fig. 1 Number of papers in which each screening method of the *GJB2/GJB6* genes and mitochondrial variants was used. The same paper may have used more than one technique

found to be 6 in 1240. In 2004, Oliveira et al. conducted a study to determine the c.35delG carrier frequency in Brazilian samples with different ethnic backgrounds. In Brazilians with a predominantly European background, the carrier frequency was 2%, in the group with reported African ancestry, it was 1%, but no c.35delG carriers were detected among 107 Asian Brazilians. In 2007, the same group of researchers expanded their sample to 1,856 randomly selected newborns from many different regions of Brazil (North, Northeast, South, and Southeast). Since the frequencies do not differ significantly between regions, they were grouped, resulting in an overall Brazilian c.35delG carrier frequency of 1.35% (25/1856). A similar study, to determine the frequency of the c.35delG and m.1555A > G, was conducted in Argentina with 712 samples of unrelated healthy blood donors and 330 unselected newborn dried blood spots, using PCR–RFLP. The c.35delG was present in 1.5% (11/712), or 1 in 65 individuals, in heterozygosis, among the healthy blood donors. Thus, the frequency is lower than the observed among the European parental populations that originated the Argentinians (Gravina et al. 2007).

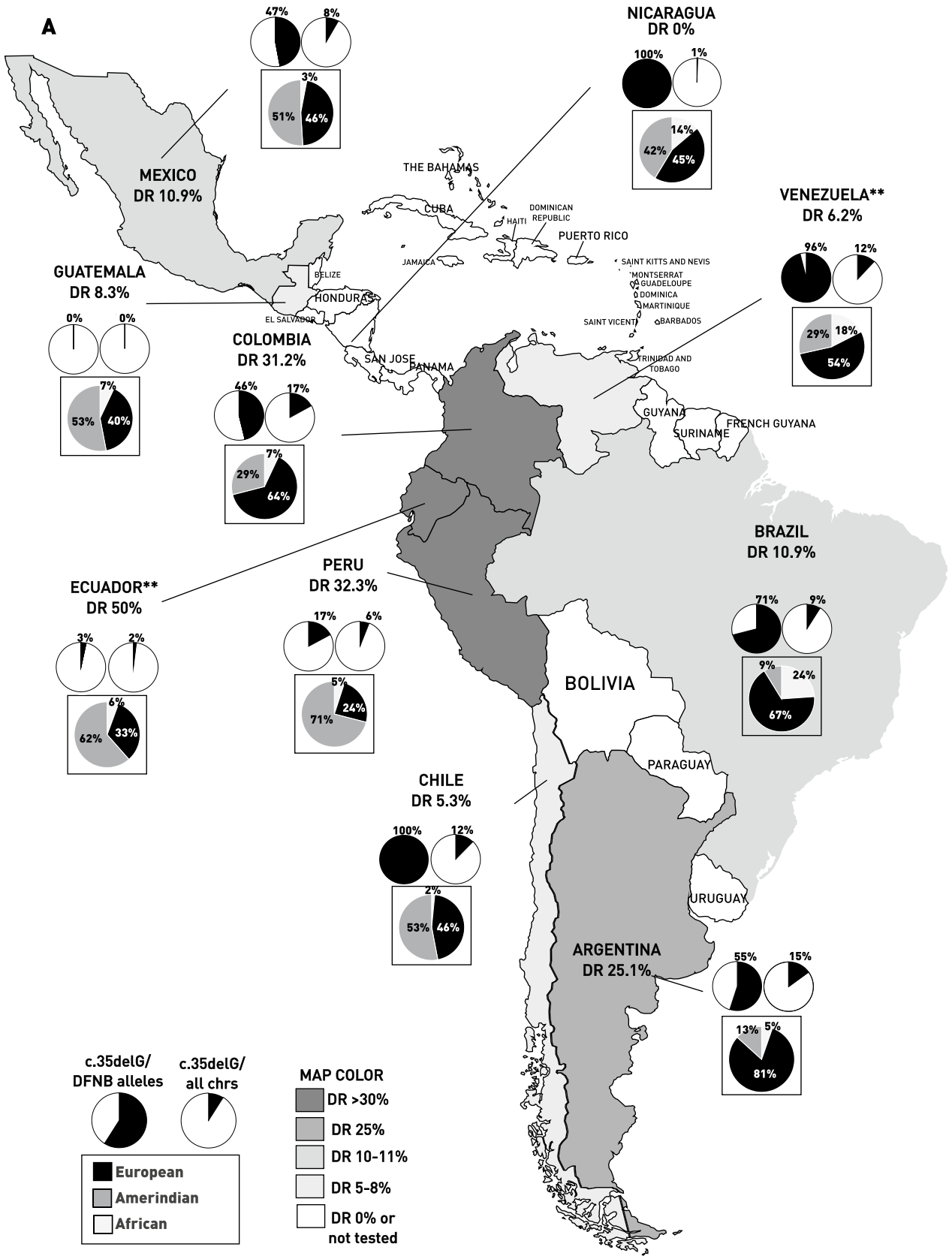
A summary of the main findings, the inclusion criteria, and the methods used for screening the *GJB2/GJB6* genes in hearing-impaired subjects are shown in Table 1 and Supplementary Table S1, which were built to allow comparison of the main results. Figure 1 presents the numbers of papers using the different methods of screening, both of *GJB2/GJB6* and mitochondrial variants. Figures 2A, B display a map of Latin America and Brazilian regions, respectively, showing *GJB2/GJB6* diagnostic rate (DR), ancestry, and the c.35delG variant frequency related to mutated alleles and all screened chromosomes. Figure 2C shows the proportions of main parental ancestries (European, African, and Native American) and the DR in the different countries/regions. Supplementary Table S2 shows the selected papers in each region, because of more negligible risk of bias, used to determine the average DRs. Finally, supplementary Table S3 provides an overview of the proportion of the three main parental ancestries in Latina American countries or regions, as obtained from different publications, used to build the Fig. 2A–C.

The diversity of alleles encountered in all studies is exhibited in Fig. 3 and summarized in Supplementary Tables S4. Table S5 shows the diversity of genotypes.

The Latin American studies confirmed the importance of *GJB2* variants as a frequent cause of NSSNHL (non-syndromic sensorineural hearing loss) and gave further support for the pathogenicity of many *GJB2* variants. The contribution of c.35delG as a causative variant was estimated in Brazil (States of São Paulo, Paraná, Minas Gerais, Rio de Janeiro, Bahia, and Pará) as well as in other countries such as Colombia, Argentina, Chile, Guatemala, Venezuela, and Mexico, in cohorts of hearing-impaired individuals (Table 1, Fig. 2A, B). Given limited funding for research and public health in many developing countries, many investigations focused only on screening the c.35delG variant, and some included the *GJB6* deletions. However, the ones which included sequencing of the whole coding region of *GJB2* revealed a wide diversity of genotypes and alleles and could reveal that other variants could play a similar role in other populations (Fig. 3, Supplementary Tables S4 and S5).

In the city of São Paulo, the most populated and multiethnic city of South America, the diagnostic rate of *GJB2/GJB6* screening was estimated as 12% (36/301) and 13.3% (72/542) in studies published in 2009 and 2021, respectively (Batissoco et al. 2009a, 2021, this issue). The cohorts described in Batissoco et al. (2009a,b) and Batissoco et al. (2021, this issue) were ascertained independently and did

Fig. 2 **A**, **B** Maps of Latin American countries and Brazilian regions, respectively, displaying the *GJB2/GJB6* Diagnostic rate (DR) and the frequency of the c.35delG variant among mutated chromosomes (the black part in the left pie chart) and all chromosomes tested (black part right pie chart). Studies that were biased towards related subjects, familial or recessive cases, were not included in studies from the same country. The *GJB2* coding region was sequenced in at least part of the patients in the included studies, but not all tested the *GJB6* deletions. The darker the grey, the higher the DR, whereas the lighter the grey, the lowest the DR. **biased towards related probands. *small sample, only one study; **C** comparison of the proportion of each parental ancestry among the different regions or countries of Latin America and the corresponding *GJB2/GJB6* DR


A
MEXICO
DR 10.9%
47%
8%
3%
51%
46%
NICARAGUA
DR 0%
100%
1%
14%
42%
45%
THE BAHAMAS
CUBA
HAITI
DOMINICAN REPUBLIC
PUERTO RICO
JAMAICA
SAINT KITTS AND NEVIS
MONTSERRAT
GUADELOUPE
DOMINICA
MARTINIQUE
SAINT VICENT
BARBADOS
TRINIDAD AND TOBAGO
VENEZUELA**
DR 6.2%
96%
12%
29%
18%
54%
GUATEMALA
DR 8.3%
BELIZE
HONDURAS
EL SALVADOR
0%
0%
7%
53%
40%
COLOMBIA
DR 31.2%
SAN JOSE
PANAMA
46%
17%
7%
29%
64%
GUYANA
SURINAME
FRENCH GUYANA
BRAZIL
DR 10.9%
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67%
ECUADOR**
DR 50%
3%
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62%
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PERU
DR 32.3%
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5%
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BOLIVIA
PARAGUAY
CHILE
DR 5.3%
100%
12%
2%
53%
46%
URUGUAY
ARGENTINA
DR 25.1%
55%
15%
13%
5%
81%
c.35delG/
DFNB alleles
c.35delG/
all chrs
European
Amerindian
African
MAP COLOR
DR >30%
DR 25%
DR 10-11%
DR 5-8%
DR 0% or
not tested

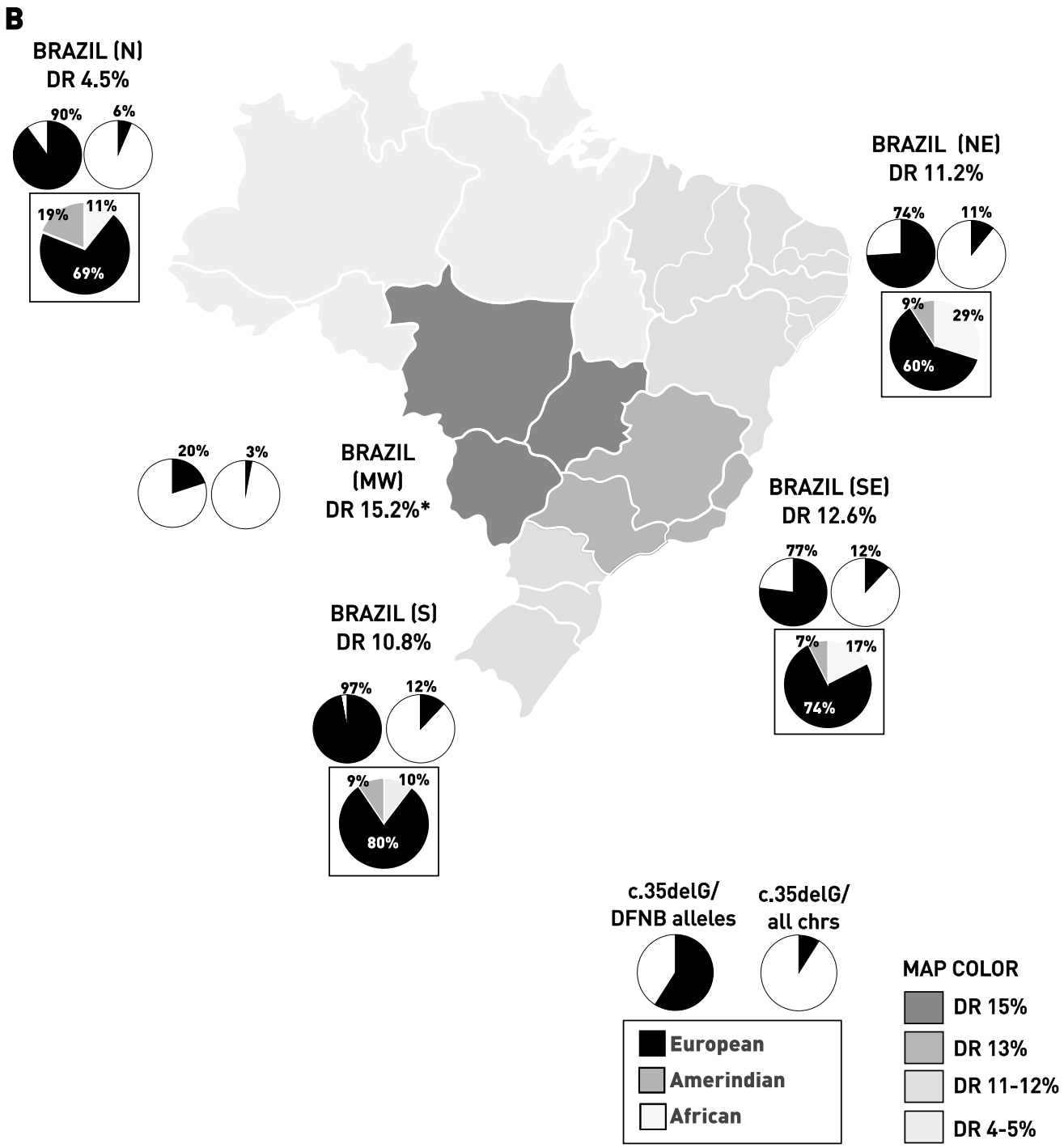


Fig. 2 (continued)

not overlap. In both studies, individuals with non-syndromic hearing loss from the state of São Paulo and different country regions were ascertained, but, in the second study, a higher proportion of probands from different regions of Brazil was included. Therefore, they can be considered rough estimates of the average Brazilian *GJB2/GJB6* contribution to the etiology of hearing loss. According to their Brazilian region of origin, the 542 subjects reported in Batissoco et al. (2021, this issue) were separated in Table 1, and thus provide a picture of the differences between the Brazilian regions.

Given the different subjects selection criteria (Table 1 and Supplementary Table S1), as well as the inclusion of already known positive cases, the studies performed near the city of Campinas (also in the state of São Paulo, Brazil) exhibited

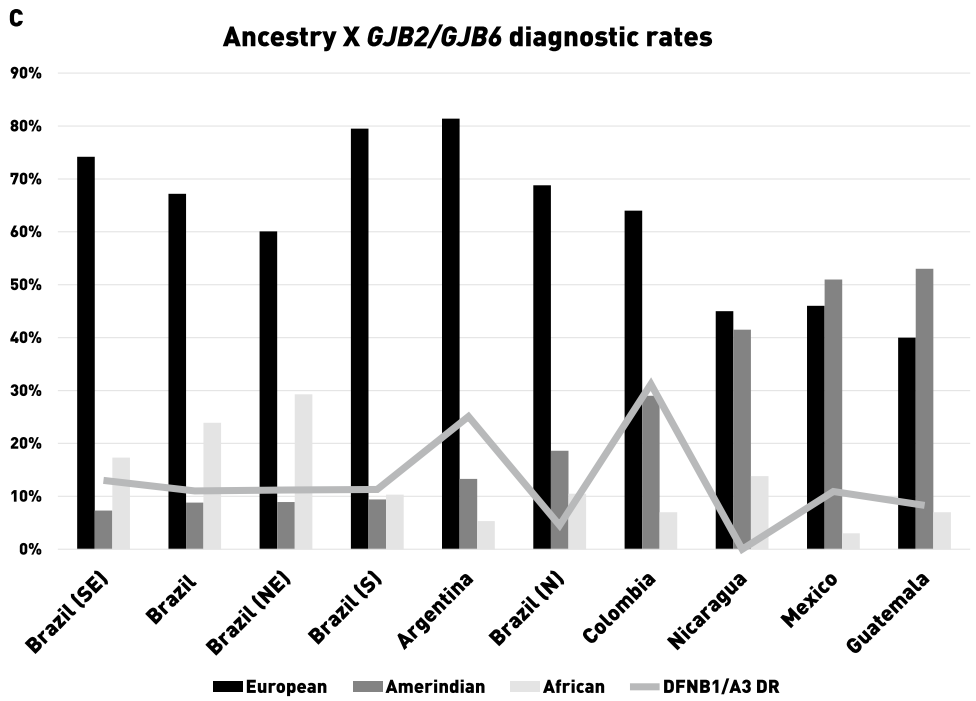


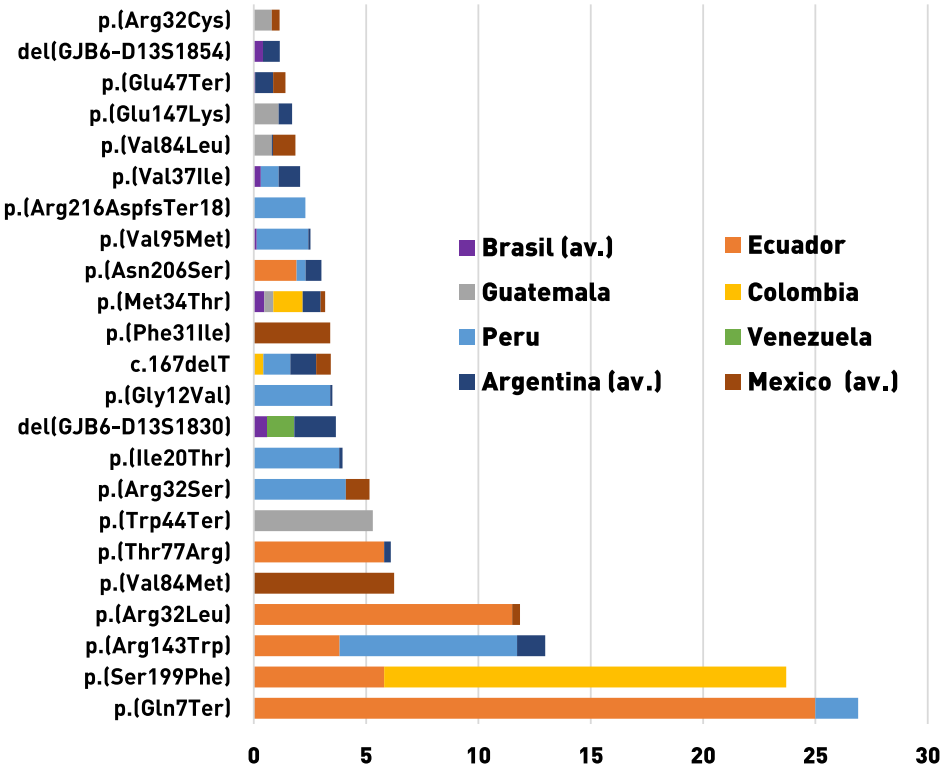
Fig. 2 (continued)

a more comprehensive range of proportions of *GJB2/GJB6* DR: 11.29% (7/62), 7.13% (46/645) and 21.3% (60/282) (Oliveira et al. 2002, 2007a,b; Martins et al. 2013). Interestingly, in the Minas Gerais State from the Southeastern Region of Brazil, none of the 53 cases of NSSNHL could be attributed to c.35delG or *GJB6* deletions, but 9 heterozygous samples with c.35delG were found (Schüffner et al. 2020). The second mutated allele was not searched through sequencing. In the other two states from the Southeastern region of Brazil, Rio de Janeiro, and Espírito Santo, *GJB2/GJB6* causative variants explained 4% and 5.2% of cases of HL, respectively (Felix et al. 2014; Cordeiro-Silva et al. 2011). However, in the study conducted in Rio de Janeiro (Felix et al. 2014), the inclusion criteria were "c.35delG negative or heterozygous", but the number of homozygous with the c.35delG was not mentioned preventing the calculation of the overall contribution of *GJB2/GJB6* and c.35delG (Table 1 and Supplementary Table S1).

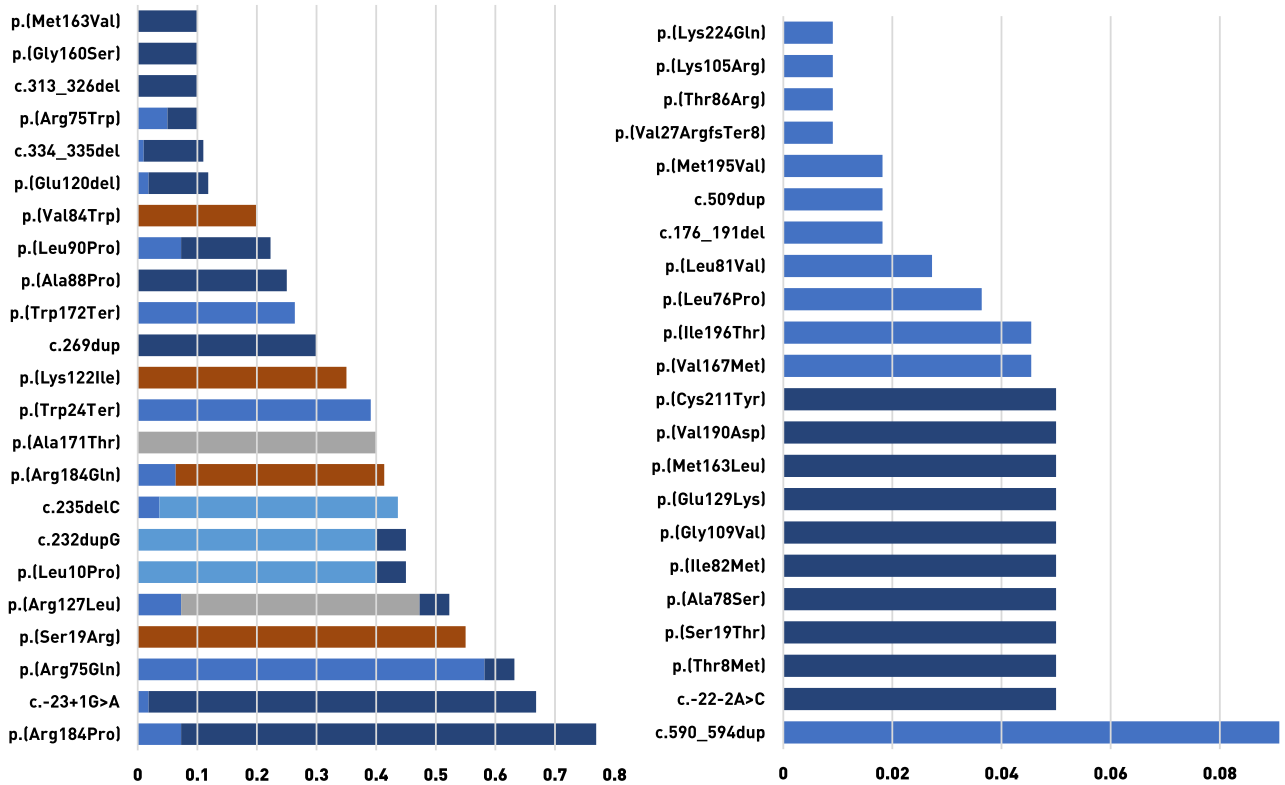
The State of Bahia is located in the Northeastern region of Brazil, and nearly 75% of its population is self-identified as having African ancestry (Supplementary Table S3). In one study from the state of Bahia (city of Monte Santo), Manzoli et al. (2013) selected probands mainly because of a positive family history of hearing loss and found a rate of *GJB2/GJB6* pathogenic variants in 37% of the cases (Table 1, Supplementary Tables S1, S4, and S5). The c.35delG variant was present in 24.7% of the screened chromosomes (40/162) and represented 80% of *GJB2/GJB6* mutated alleles. However, this proportion may be overestimated since the study included related probands, likely explaining the high proportion of diagnoses. In addition, more than half of the *GJB2* homozygotes were born from consanguineous marriages (Table 1, Supplementary Tables S1). Those frequencies are comparable to those observed in a study from the Colombian isle of Providencia, which also included related probands, yielding a proportion of *GJB2/GJB6* causative variants or c.35delG of 47% (Lattig et al. 2008). Thus, founder effects and high rates of consanguinity may explain the high contribution of the c.35delG to NSHL in this island (Tables 1 and Supplementary Table S1).

In the rural settlements of Queimadas e Gado Bravo, in the Northeastern Brazilian state of Paraiba, the frequency of *GJB2/GJB6* variants and c.35delG observed were similar to those observed in the city São Paulo, probably reflecting the unbiased sample selection, as shown in Table 1 (also in Supplementary Tables S1, S2, S4, and S5; Melo et al. 2014). Amazingly, in Belém city from the Amazonian region, the *GJB2/GJB6* diagnostic rate was about one-tenth of the average Brazilian rate, since only one case with c.35delG in the homozygous state was observed among 77 probands with HL (Table 1, Supplementary Tables S1, S2, S4, and S5), suggesting that other genes might be more relevant as causing HL in this population (Castro et al. 2013), as well as environmental factors. It is important to highlight that in the Amazonian region, the Native American genetic

A ***GJB2/GJB6*** **most frequent variants**



B ***GJB2/GJB6*** **less frequent variants**



◂**Fig. 3** Diversity and frequencies of variants (protein description) in *GJB2/GJB6* genes among Latin American countries; c.35delG was excluded, and the variants were divided into three charts to allow a better analysis; **A** other frequent variants and **B** less frequent variants (colors of each country are the same in **A** and **B**, except for Brazil which is purple in A and medium blue in B)

contribution is the highest in the country, which might explain the rarity of c.35delG (Supplementary Table S3, Fig. 2B, C).

As more restricted selection criteria are included in the study, the higher the *GJB2/GJB6* contribution, as expected, especially in the samples with a significant European ancestry (mainly Spanish) (Table 1, Fig. 2A–C, Supplementary Tables S1, S2, and S3). For instance, in an Argentinian sample composed of cases with prelingual NS-SNHL, after environmental factors, malformations, or autosomal dominant inheritance was excluded, the DR was 34%, thus approaching the 50% estimate of *GJB2/GJB6* contribution to AR NSSNHL in European populations (Gravina et al. 2010). A high diagnostic rate was also observed in Peru, 32.4%, in a sample with a bias towards familial cases (Table 1, Fig. 2A, C and Supplementary Table S1), but without c.35delG as the most common variant (Table 1, Fig. 2A, and Supplementary Table S4, Figueroa-Ildefonso et al. 2019). On the other hand, in a sample with similar inclusion criteria, enriched towards familial and presumably autosomal recessive cases, but in Venezuela, a lower DR was obtained (4.8–7.5%), probably due to minor European contribution to its genetic background (Table 1, Fig. 2A, C, Supplementary Tables S1, S2, and S3; Angeli et al. 2000; Utrera et al. 2007).

A different approach for estimating *GJB2* and c.35delG contribution to HL was employed by Nivoloni et al. (2010), who screened the c.35delG mutation in 8974 unselected newborns who also underwent audiological testing (transient otoacoustic emissions). Among 17 patients who failed in the audiological testing, 4 were homozygotes with c.35delG, thus explaining 23.5% of the HL cases. They also found 84 cases of c.35delG heterozygotes who passed the audiological exams. The authors mentioned that these heterozygotes would have their hearing followed. However, sequencing of *GJB2* was not reported to clarify if those subjects had a second mutated allele and would have a higher probability of developing a hearing impairment.

In the paper in which the second deletion involving *GJB6* del(*GJB6*-D13S1854) was described, a multinational cohort including subjects from Brazil was screened, and it was found to account for 6.3% of the affected *GJB2* heterozygotes (Del Castillo et al. 2005). Both most common deletions, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), are also among the most frequent variants in Latin America, the first in Argentina, Venezuela, and Brazil, and the last in Argentina and Brazil (Fig. 3A; Supplementary Table S4 and S5).

The frequency of pathogenic *GJB2* variants among the different populations that compose Latin America was related to the admixture proportions in each of them (Fig. 2A–C, Supplementary Tables S2 and S3). The populations with a higher European contribution tend to have higher diagnostic yields when *GJB2* is investigated (Fig. 2C). The higher the proportion of Amerindian contribution, the lower the frequency of *GJB2* causative variants found among the hearing impaired since the high diagnostic rates of *GJB2/GJB6* related hearing loss is mainly due to the c.35delG variant. The influence of ancestry in the frequency of disease was also stated by Fejerman et al. (2010), which found an association between European ancestry and risk of breast cancer in Latin American women while studying Mexican women. In populations with reduced European contribution, it is possible that other genes, yet unidentified or not frequently screened, have a more substantial contribution as a cause of hearing loss. Besides ancestry, another factor to consider when interpreting the frequencies displayed in Fig. 2, as influencing results of molecular diagnosis, is the fraction of the population that has access to it, because it is well known that health care is not equally available, and some populations or groups might not reach the Genetic Services. For instance, there are remarkable differences in parental ancestry when people from low and high socioeconomic levels are compared in Venezuela, with 40% and 17% of Amerindian ancestry and 33% and 75% of European ancestry, respectively (Martínez et al. 2007).

Regarding *GJB2* recessive variants, it is interesting to observe some likely consequences of founder effects in each Latin American region analyzed. Some variants have high frequencies in some regions and are rare or non-existent in other locations. For instance, c.590_594dup: p.(Ser199GlnfsTer9) was only described in Rio de Janeiro city, the c.131G > A: p.(Trp44Ter) variant was observed in a high frequency in Guatemala but was not observed in the other Latin American countries. The same trend was observed for the c.596C > T: p.(Ser199Phe) variant in Bogotá (Colombia) and Ecuador, for the variant c.19C > T: p.(Gln7Ter) in Ecuador and for the variant c.94C > A: p.(Arg32Ser) in Lima (Peru) and Mexico. The variants c.269dup: p.(Val91SerfsTer11), c.229 T > C: p.(Thr77Arg), c.246C > G: p.(Ile82Met), c.487A > C: p.(Met163Val) were detected only in the Argentinian samples, and p.Lys122Ile only in Mexico (Fig. 3A, B; Supplementary Table S5).

Studies from Latin America also contributed to the description of novel pathogenic variants in *GJB2*:c.487A > C: p.(Leu76Pro) (Batissoco et al. 2009b), c.79_82delinsAGA [p.(Val27ArgfsTer8)] described in Batissoco et al. (2021, this issue), c.314A > G: p.(Lys105Arg) described by Oliveira et al. (2007b), c.c.29 T > C: p.(Leu10Pro) and c.326G > T: p.(Gly109Val) reported by Dalamón et al. (2010), c.35G > A: p.(Gly12Asp) by Hernández-Juárez

et al. (2014), c.590_594dup: p.(Ser199GlnfsTer9) by Felix et al. (2014).

Regarding the dominant pathogenic variants associated with variable epidermal and nails symptoms, such as keratoderma palmoplantar or nail dysplasia, the most frequently observed variant was p.(Arg75Gln), followed by p.(Arg75Trp) and p.(Arg184Gln). In addition, a Cuban family with sensorineural congenital profound HL in which a novel dominant variant was segregating was described (NM_004004.6: c.61G > A, p.(Gly21Arg) (Rabionet et al. 2006). This variant is neither listed in the Deafness Variation Database nor ClinVar.

Martínez-Saucedo et al. (2015) described two Mexican families in which six different recessive *GJB2* pathogenic variants were segregating, and the affected members were compound heterozygous with three pathogenic variants in *GJB2* (Table 1). The p.(Ser19Arg) was in the same chromosome as p.(Arg32Ser), both in *trans* with p.(Glu47Ter) in the first family. Likewise, in the second family, the p.(Phe31Ile) was in the same chromosome of p.(Val84Met), both in *trans* with p.(Trp44Ter). To date, p.(Ser19Arg) was detected only in the Mexican population (previous report from the same group), in which p.(Phe31Ile) was also reported (Supplementary Table S4). The p.(Phe31Ile) variant has only been described in one additional study of an extensive North American cohort (Putcha et al. 2007). Conversely, p.(Val84Met) was also reported in this previous publication (Loeza-Becerra et al. 2014), of the same group (Supplementary Table S4), but also in other populations, for instance, in Portugal (Matos et al. 2015) and China (Li et al. 2014). Furthermore, the p.(Arg32Ser) variant was reported in Peru and Mexico in a paper from the same group (Loeza-Becerra et al. 2014) as well as in the Japanese population (Hayashi et al. 2011). Thus, a plausible hypothesis is that these two less frequent variants, p.(Ser19Arg) and p.(Phe31Ile), arose in chromosomes that already carried the p.(Arg32Ser) and p.(Val84Met) variants, respectively. This study exemplifies the complexity of *GJB2* mutational patterns, together with the diversity of variants found in Latin America.

The case of "Monoallelic" Variants

Data from several countries demonstrate a significant proportion of *GJB2/GJB6* monoallelic patients with hearing loss, with a single recessive pathogenic variant detected, even after screening for the noncoding *GJB2* variant (c.-23 + 1G > A) or *GJB6* deletions. For example, in a recent and complete investigation performed by Batissoco et al. (2021 this issue), the rate of monoallelic patients among those bearing *GJB2/GJB6* pathogenic variants was 16/88 (18.2%).

Even though not all studies performed all available methods for testing *GJB2/GJB6* variants, some reported a high frequency of monoallelic subjects, including heterozygotes bearing rare variants, making it challenging to explain the occurrence of all cases by chance alone. For instance, Oliveira et al. (2007b) found 22 subjects monoallelic to *GJB2* pathogenic variants (31.4%; 22/70). Felix et al. (2014) also found a high rate of monoallelic patients, 84.2% (16/19), but the selection criteria for recruitment was "c.35delG negative or heterozygous patients". Interestingly, there were heterozygotes with rare pathogenic variants, such as c.590_594dup: p.(Ser199Glnfs*9), c.587 T > C: p.(Ile196Thr), c.380G > T: p.(Arg127Leu) and c.499G > A: p.(Val167Met) in addition to 13 c.35delG heterozygotes. Castro et al. (2013) observed a similar frequency of monoallelic cases, 77.8% (7/9). Unfortunately, *GJB6* deletions and the noncoding pathogenic *GJB2* variants (for instance, c.-23 + 1G > A and c.-22-2A > C) were not screened in their study, as in many of the other studies conducted in Latin America, which could potentially solve some of these cases. For example, da Silva-Costa et al. (2009) screened the c.-23 + 1G > A in 185 unrelated Brazilians with hearing loss, 43 heterozygous with *GJB2* variants and the remaining without variants detected and identified 2 cases presenting the splicing variant (c.-23 + 1G > A) in compound heterozygosis with c.35delG (4.6% of the monoallelic cases).

On the other hand, Figueroa-Ildefonso et al. (2019) found only 5.3% of monoallelic individuals in a sample of 133 HL patients from the Peruvian city of Lima. In a sample of 26 probands from 11 families from Ecuador, 11.5% were found to be monoallelic, and no *GJB6* deletion was detected (Paz-y-Miño et al. 2014). Thus, on average, monoallelic *GJB2/GJB6* genotypes represented 38% of the cases of hearing loss investigated, ranging from 5.3 to 84.2% of cases. NGS sequencing, either of gene panels or the whole exome, has been demonstrated to solve at least part of these cases, for example, 22% of them, in an investigation led by Pang et al. (2014) in a Han Chinese sample. In agreement with the hypothesis that part of the monoallelic subjects should have another etiology for HL, among the 16 *GJB2* monoallelic cases described in Batissooco et al. (2021, this issue), 1 was also clinically diagnosed with Waardenburg Syndrome, and a second patient presented with Mondini dysplasia plus EVA.

In conclusion, it is likely that the proper search for *GJB6* deletions and noncoding variants in *GJB2*, associated with NGS of other HL genes, will clarify the etiology of a significant amount of monoallelic cases, and the remaining ones are possibly the result of the occurrence of variants in heterozygosis, by chance.

Mitochondrial variants contribution to NSHL in Latin American populations

Since its first report by Prezant et al. (1993), m.1555A > G in *MT-RNR1* was revealed to be the most frequent variant associated with non-syndromic hearing loss, with mitochondrial

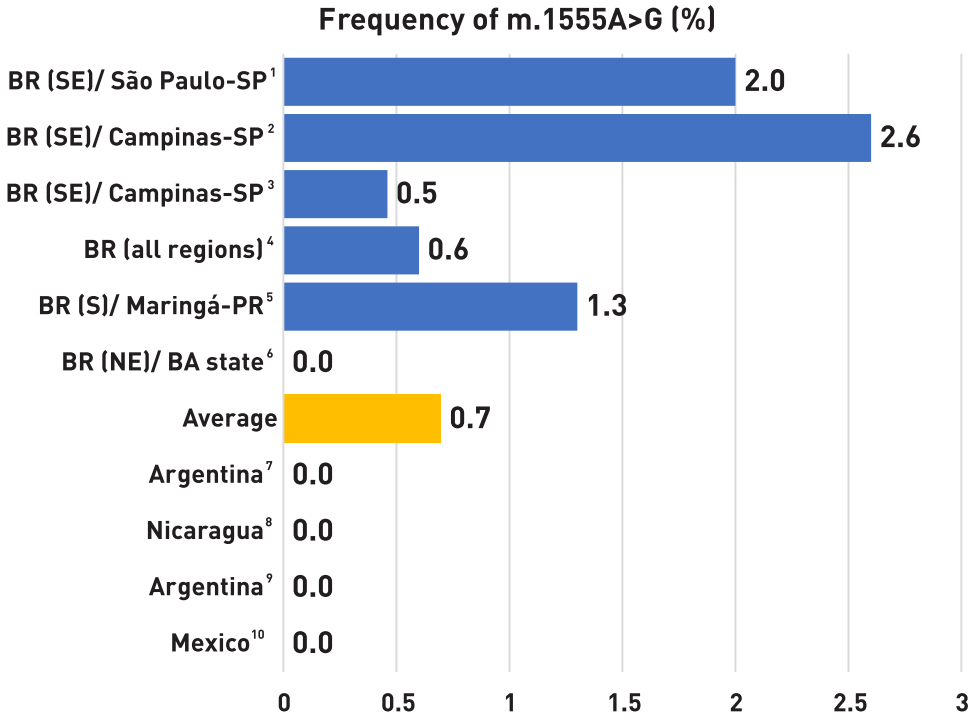


Fig. 4 Frequency of the m.1555A > G variant among the Latin American studies.[1]Abreu-Silva et al. (2006a; b), [2]Alves et al. (2016), [3]Oliveira et al. (2007b), [4]Batissoco et al. (2021), [5]Salomão et al. (2013), [6]Manzoli et al. (2013), [7]Dalamón et al. (2010), [8]Saunders et al. (2009), [9]Gravina et al. (2007), [10]Arenas-Sordo et al. (2012)

transmission. Moreover, in the definitive study of Estivill et al. (1998a), the onset and severity of hearing loss due to m.1555A > G were strongly influenced by treatment with ototoxic antibiotics of the group of the aminoglycosides.

The methods used to screen the mitochondrial variants in Latin American studies retrieved in our search are shown in Fig. 1.

The first investigation about mitochondrial variants in Latin America was the study of Abreu-Silva et al. (2006a; b), who found that 2% of their cohort of 203 NSHL subjects, ascertained in the state São Paulo (Brazil), presented the m. 1555A > G variant (Fig. 4). Other hearing loss-related variants in the tRNASer(UCN) gene (*MT-TS1*) were also screened, but with negative results. In one of the cases with m.1555A > G, there was documented evidence of exposure to aminoglycosides anticipating the HL onset and increasing its severity (Pupo et al. 2008). Another independent study performed in São Paulo, but with patients originating from many Brazilian regions, Batissoco et al. (2021, this issue) found a lower frequency of this variant, 0.6% (3/542); in one case, there was aminoglycoside exposure before HL onset. The different frequencies of m.1555A > G variant encountered by Abreu-Silva et al. (2006a, b) and Batissoco et al. (2021 this issue), 2% versus 0.6%, in the same city, might be partially explained by the fact that these studies were performed 15 years apart. After 2011, strict control in the use of antibiotics was implemented in Brazil (law RDC nr 20 May 5th, 2011), which might have contributed to a decrease in the penetrance of m.1555A > G. Those observations highlight the positive effects of public health normalizations in broader aspects that are anticipated.

Oliveira et al. (2007b) screened the m.1555A > G and m.7445A > G mitochondrial variants in the *MT-RNR1* gene in 207 probands with HL from Campinas city, Brazil. The m.7445A > G variant had been previously associated with syndromic hearing loss (Palmoplantar keratoderma; Sevior et al. 1998). They found two cases bearing m.1555A > G (0.46%), with reported association with aminoglycosides. Alves et al. (2016) screened 152 unrelated patients with moderate to profound sensorineural HL, as well as 104 samples from normal hearing individuals, also from Campinas city, searching for mitochondrial variants through iPLEX Gold/MALDI-TOF MS technology. The m.1555A > G pathogenic variant was present in 4/152 (2.6%) of the affected group and absent in the unaffected. Salomão et al. (2013), in Maringá city from the Brazilian state of Paraná, encountered one case presenting with prelingual HL (1.3%) with the variant m.1555A > G, in a cohort of 78 HL subjects.

On the other hand, m.1555A > G was not detected in samples from Bahia State, Brazil (81 subjects; Manzoli et al. 2013) and 2 studies from Argentina with 252 individuals (Dalamón et al. 2010) and with 1042 individuals (Gravina et al. 2007), and from Mexico (76 probands, Arenas-Sordo et al. 2012). Saunders et al. (2009) sequenced the 12S rRNA (*MT-RNR1)* gene in 31 deaf children with childhood or uterus exposure to gentamicin from rural Nicaragua, but no pathogenic variants were identified.

There were two reports in which a possible association between mitochondrial variants, m.1291 T > C and

m.827A > G, and HL was suggested (Ballana et al. 2006; Chaig et al. 2008). However, a proper matching of the affected subjects and the controls demonstrated no difference in the frequencies of these variants among the two groups (Abreu-Silva et al. 2006b; Uehara et al. 2010), suggesting that the pathogenicity of both variants is questionable.

Adding the results of all studies (Abreu-Silva et al. 2006a; b; Oliveira et al. 2007b; Salomão et al. 2013; Alves et al. 2016; Batissoco et al. 2021; Manzoli et al. 2013; Dalamón et al. 2010; Gravina et al. 2007; Arenas-Sordo et al. 2012; Saunders et al. 2009) the conclusion is that near 0.7% of all HL cases screened in Latin America were due to the presence of the m.1555A > G variant (Fig. 4). There is little information regarding the presence of other mitochondrial mutations. For genetic counseling purposes, it is highly relevant that the m.1555A > G variant should be screened in Latin American countries, even in unselected cases of HL, since most genetic tests employed to detect it are cheap and straightforward. Furthermore, in the pedigrees in which it is segregating, prevention is possible through the warning about the role of ototoxic agents in the onset of hearing loss.

OTOF contribution to ARNSHL and auditory neuropathy in Latin American populations

Pathogenic variants in the *OTOF* gene were first reported as a cause of autosomal recessive sensorineural hearing loss (ARNSHL) in four unrelated Lebanese families (Yasunaga et al. 1999). In 2003, Varga et al. established the relationship between auditory neuropathy and *OTOF* pathogenic variants. Auditory neuropathy is a type of sensorineural hearing loss characterized by an absent or abnormal auditory brainstem response (ABR), with preservation of otoacoustic emissions (OAEs), and/or cochlear microphonics (CMs).

Some studies focused on screening variants in *OTOF* in unselected hearing-impaired patients. However, due to the reported association of *OTOF* with the auditory neuropathy phenotype, many studies introduced auditory neuropathy as a selection criterion for samples to be submitted to the sequencing of the whole coding region of *OTOF* by the Sanger method, mainly before the availability of massive parallel sequencing. Variants in *OTOF* found in Latin American studies are presented in Table 2, after reevaluation according to recent ACMG criteria. Table 2 also presents the reevaluation of nomenclature and the pathogenicity status of the variants described in the study of Romanos et al. (2009), after reassessment of some cases with massive parallel sequencing and ACMG classification, in addition to eight cases more recently ascertained in the same laboratory, in São Paulo, Brazil. All variants were submitted to ClinVar. Figure 5A illustrates the methods used to identify these variants, and Fig. 5B shows the frequency of the different variants among the countries. Figure 6 displays the pedigrees in which the variants described in the present study are segregating.

The variant c.2485C > T:p.(Gln829Ter), usually known as Q829X, is a frequent pathogenic variant in *OTOF*, firstly identified in the Spanish population with a frequency of 4.4% in cases of AR-NSHL (Rodríguez-Ballesteros et al. 2003; Migliosi et al. 2002). However, it appears to be less frequent in Latin America. It has been detected in one Mexican (Varga et al. 2006) and two Argentinian patients (Reynoso et al. 2004). Rodríguez-Ballesteros et al. (2008) studied 83 Colombian and 30 Argentinean subjects and also screened for the variant c.2485C > T:(p.Gln829Ter) in the *OTOF* gene. Homozygosity was identified in one Colombian patient. Besides, one compound heterozygote [p.(Gln829Ter)/p.(Arg708Ter)] from Colombia and two from Argentina [p.(Gln829Ter)/c.2905_2923delinsCTCCGAGCGCA] were also identified in their study. However, (p.Gln829Ter) was not detected in hearing-impaired cohorts from the Brazilian cities of Campinas (207 subjects, Oliveira et al. 2007b) and São Paulo (342 subjects, Romanos et al. 2009). Carvalho et al. (2016a,b) investigated the p.(Gln829Ter) variant in 47 patients with auditory neuropathy, with varying ages of onset, also from Campinas city. None of them showed p.(Gln829Ter), but three of them were found to be homozygous for the c.35delG variant, further emphasizing the relevance of screening this variant in any case of hearing loss, regardless of the clinical presentation. The other exons of *OTOF* were not screened to rule out pathogenic variants in this study. Remarkably, p.(Gln829Ter) was seen only in Spanish-speaking countries and never in Brazilian cities, where Portuguese ancestry is much more prominent than Spanish.

Silva et al. (2015) screened five different *OTOF* variants [c.2416 T > A: p(Y730Ter), c.766-2A > G, c.2485C > T: p.(Gln829Ter), c.5473C > G: p.(Pro1825Ala) and c.3032 T > C: p.(Leu1011Pro)] in a sample composed of 16 index cases selected because of auditory neuropathy, from the city of São José do Rio Preto, in the state of São Paulo, Brazil. One proband was heterozygous with c.766-2A > G, and five were heterozygous with c.5473C > G: p.(Pro1825Ala), and two probands, who carried two of the five variants, were identified. However, the sample from the normal hearing mother of one of these subjects also showed both variants, indicating that they are in *cis*.

From a cohort of 342 Brazilian hearing-impaired patients, 48 probands with presumptive autosomal recessive inheritance (born from consanguineous or equally affected sibs and unaffected parents), 4 familial and 7 auditory neuropathy isolated cases were selected for *OTOF* investigation (Romanos

Table 2 OTOF variants described in Latin America

Country/City	Reference: Study design	Methods	Family Proband nr	Genomic position (GRch37)	Updated variant description HGVS (*OTOF*, NM_194248)	Other genes	Consequence	Copies	Clin Var/DVD	dbSNP	Pop. Freq. (%)	ACMG Criteria and Classification	ACMG criteria reanalysis
Brazil/São Paulo	Romanos et al. (2009): Screening of p.(Gln829Ter) in 342 subjects; Linkage: 48 AR cases, 4 familial AN and 7 sporadic AN; Sanger seq.: 11 cases compatible with linkage to *OTOF*	RFLP + Linkage (micros.) + Sanger	1	2:26,698,850:CGGCAAAGAGGCTGCGGGC > TGCGCTCGGAG	c.2905_2923delinsCTCCGAGCGCA: p.(Ala969LeufsTer30)	–	Frameshift	Hetero	P/P	rs397515596	n.l.	**LP** (PVS1, PM2)	Variant nomenclature, still inconclusive (monoallelic)
			Simplex 2*	2:26,705,285:TCATTAGAAATCTTGCG > T	c.1552_1567del: p.(Arg518ThrfsTer15)	–	Frameshift	Hetero	P/P	rs1443739332	0.0007*	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss (second variant detected after WES)**
				2:26,686,975:G > A	c.4961-1G > A	–	Splicing	Hetero	SCV001762954./n.l	n.l.	n.l.	**LP** (PM2, PM3, PP3, PP1)	
			3	2:26,696,867:G > A	c.3400C > T: p.(Arg1134Ter)	–	Stop_gained	Homo	P/P	rs199848801	0.0016	**P** (PVS1, PM2,PM3, PP1, PP3)	**Causative of hearing loss**
			Multiplex cons. 4*	2:26,687,737:C > T	c.4960G > A: p.(Gly1654Ser)	–	Missense	Hetero	SCV001762956 /P	rs1005694756	0.0012	**LP** (PS1, PM2, PP1, PP2, PP3)	**Causative of hearing loss (second variant detected WES)**
				2:26,688,904:T > C	c.4541A > G: p.(Asp1514Gly)	–	Missense	Hetero	SCV001762957/n.l	n.l.	n.l.	**LP** (PM2, PM3, PP1, PP2, PP3)	
			5	2:26,700,341:GC > G	c.2348del: p.(Gly783AlafsTer17)	–	Frameshift	Hetero	P/P	rs80356591	0.0039	**P** (PVS1, PS1, PM2, PP3)	**Causative of hearing loss**
				2:26,683,527:A > AG	c.5800dup: p.(Leu1934ProfsTer251)	–	Frameshift	Hetero	LP/LP	rs397515609	0.0004	**P** (PVS1, PS1, PM2,PP3)	
			6	2: 26,703,142:C > T	c.1841G > A: p.(Gly614Glu)	–	Missense	Hetero	P/P	rs397515589	0.00**	**LP** (PS1, PM2, PP3, PP2, PP1)	**Causative of hearing loss**
				2:26,697,430:C > G	c.3239G > C: p.(Arg1080Pro)	–	Missense	Hetero	P/P	rs397515598	0.0021*	**LP** (PS1, PM2, PP3, PP2, PP1)	
			multiplex 7**	2:26,686,954:C > T	c.4981G > A: p.(Glu1661Lys)	–	Missense	Hetero	SCV001762963 /P	rs894098325	0.0008	**LP** (PM2, PM3, PP1, PP2, PP3)	**Causative of hearing loss**
				2:26,684,666:T > A	c.5431A > T: p.(Lys1811Ter)	–	Nonsense	Hetero	SCV001762962./P	rs1487432642	0.0004[a]	**P** (PVS1, PS1, PM2, PP1, PP3)	
			8	2:26,683,543:T > G	c.5785A > C: p.(Asn1929His)	–	Missense	Hetero	SCV001762964 /P	rs896148523	0.0015[a]	**VUS** (PM2,PP3, PP2)	Inconclusive (monoallelic)
Brazil/ Cuiabá-MT	Batissoco et al. (2021): 542 subjects, 19 w/ AN, 1 selected with 2 affected siblings—Sanger seq.; 1 randomly selected sporadic case—NGS	Sanger	Multiplex O1	2:26,698,304:C > A	c.3049G > T: p.(Glu1017Ter)	–	Stop_gained	Hetero	SCV001762970 /n.l	n.l.	n.l.	**P** (PVS1, PM2, PM3, PP1)	**Causative of hearing loss**
				2:26,696,867:G > A	c.3400C > T: p.(Arg1134Ter)	–	Stop_gained	Hetero	P/P	rs199848801	0.0016	**P** (PVS1, PS1, PM2, PP1)	
Brazil/ Rio de Janeiro-RJ		WES	Simplex O_2	2:26,702,193:C > T	c.2153G > A: p.(Trp718Ter)	–	Stop_gained	Hetero	P/P	rs111033383	0.0024	**P** (PVS1, PS1, PM2, PP1)	**Causative of hearing loss**
				2:26,696,935:G > A	c.3332C > T: p.(Pro1111Leu)	–	Missense	Hetero	VUS/VUS	rs141972928	0.0378*	**LP** (PM2, PM3, PP2, PP3)	

Table 2 (continued)

Country/City	Reference: Study design	Methods	Family Proband nr	Genomic position (GRch37)	Updated variant description HGVS (*OTOF*, NM_194248)	Other genes	Consequence	Copies	Clin Var/DVD	dbSNP	Pop. Freq. (%)	ACMG Criteria and Classification	ACMG criteria reanalysis
Brazil/São Paulo	*Present study*: Five cases by WES, monoallelic *OTOF* cases of Romanos et al. (2009) or AN; four new cases of AN by Sanger seq., two putative cases of AR by NGS panel of 99 HL genes	Sanger	Simplex cons. 9	2:26,705,285:TCA TTAGAAATC TTGCG>T	c.1552_1567del: p.(Arg518ThrfsTer15)	–	Frameshift	Homo	P/P	rs1443739332	0.0007*	**P** (PVS1, PS1, PM2, PP1, PP3)	**Causative of hearing loss**
		Sanger	Simplex cons.10	2:26,688,621:A>G	c.4718 T>C: p.(Ile1573Thr)	–	Missense	Homo	P/LP	rs111033405	0.004	**P** (PS1, PM2, PM3, PP1, PP2, PP3)	**Causative of hearing loss**
		NGS panrl of 99 HL genes	Multiplex 11	2:26,698,882:G>T	c.2891C>A: p.(Ala964Glu)	–	Missense	Hetero	P/P	rs201329629	0.008#	**P** (PS1, PM2, PM3, PP2, PP3)	**Causative of hearing loss**
				2:26,688,592:G>A	c.4747C>T: p.(Arg1583Cys)	–	Missense	Hetero	P/P	rs781688103	0.002	**P** (PS1,PM2, PM3, PP2, PP3)	
		Sanger	Simplex cons.12	2:26,705,285:TCA TTAGAAATC TTGCG>T	c.1552_1567del: p.(Arg518ThrfsTer15)	–	Frameshift	Homo	P/P	rs1443739332	0.0007*	**P** (PVS1, PS1, PM2, PM3, PP1, PP3)	**Causative of hearing loss**
		WES	Simplex 13	2:26,702,193:C>T	c.2153G>A: p.(Trp718Ter)	–	Stop_gained	Hetero	P/P	rs111033383	0.0024	**P** (PVS1, PS1, PM2, PP3)	**Causative of hearing loss**
				2:26,698,304:C>A	c.3049G>T: p.(Glu1017Ter)	–	Stop_gained	Hetero	SCV001762970/n.l	n.l.	n.l.	**P** (PVS1, PM2, PM3, PP1, PP3)	
		Sanger	Simplex 14	2:26,705,285:TCA TTAGAAATC TTGCG>T	c.1552_1567del: p.(Arg518ThrfsTer15)	–	Frameshift	Hetero	P/P	rs1443739332	0.0007*	**P** (PVS1, PS1, PM2, PM3, PP3)	**Causative of hearing loss**
				2:26,706,329:CC>C	c.1392+1del	–	Splicing	Hetero	SCV001762971/n.l	n.l.	n.l.	**P** (PVS1, PM2, PM3, PP3)	
		WES	Simplex 15	2:26,696,125:T>C	c.3608A>G: p.(Asn1203Ser)	–	Missense	Hetero	B/B	rs61740776	0.0039	Benign (BA1, BP6, BP4, PP2)	ACMG criteria reanalysis, pathogenic variant in another gene detected
				10:123,246,872:C>A	–	*FGFR2*: NM_000141 c.2053G>T: p.(Asp685Tyr)	Missense	Hetero	SCV001762974/n.l	n.l.	n.l.	**LP** (PM1, PM2, PM6, PP2,PP3)	
		NGS PANEL OF 99 HL GENES	Multiplex 16	2:26,696,373:C>A	c.3471G>T: p.(Arg1157=)	–	Synonymous	Hetero	CI/B	rs61742191	0.1183	**LB** (BP4,BP7,PM2)	ACMG criteria reanalysis, candidate variant in another gene detected
				2:26,697,466:C>T	c.3203G>A: p.(Arg1068His)	–	Missense	Hetero	SCV001762973/ VUS	rs180748688	0.0152	**VUS** (PM1,PM2, PP3)	
				2:133,198,275:T>C	–	*P2RX2*: NM_174873: c.1133 T>C: p.(Phe378Ser)	Missense	Hetero	SCV001762975/n.l	rs143626910	0.004	**VUS** (PM1, PP3)	

Table 2 (continued)

Country/City	Reference: Study design	Methods	Family Proband nr	Genomic position (GRch37)	Updated variant description HGVS (*OTOF*, NM_194248)	Other genes	Consequence	Copies	Clin Var/DVD	dbSNP	Pop. Freq. (%)	ACMG Criteria and Classification	ACMG criteria reanalysis
Colombia	Rodríguez-Ballesteros et al. (2008): Screening of p.(Gln829Ter), followed by Sanger seq—83 Colombian AR cases, 2 w/ biallelic variants, one of which later diagnosed with AN	RFLP + Sanger	simplex 13NS	2:26,700,078:G > A	c.2485C > T: p.(Gln829Ter)	—	Stop_gained	Homo	P/P	rs80356593	0.0172	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss**
			multiplex 32NS	2:26,700,078:G > A	c.2485C > T: p.(Gln829Ter)	—	Stop_gained	Hetero	P/P	rs80356593	0.0172	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss**
				2:26,702,224:G > A	c.2122C > T: p.(Arg708Ter)	—	Stop_gained	Hetero	P/P	rs80356590	0.0306#	**P** (PVS, PS1, PM2, PM3, PP1)	
Argentina	Rodríguez-Ballesteros et al. (2008): Screening of p.(Gln829Ter), followed by Sanger seq—30 Argentinian AR cases + AR AN cases. Among the biallelic cases, three confirmed AN	RFLP + Sanger	simplex AEF05	2:26,700,078:G > A	c.2485C > T: p.(Gln829Ter)	—	Stop_gained	Hetero	P/P	rs80356593	0.0172	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss**
				2:26,698,850:CGGCAAAGAGGCTGCGGGC > TGCGCTCGGAG	c.2905_2923delinsCTCCGAGCGCA: p.(Ala969LeufsTer30)	—	Frameshift	Hetero	P/P	rs397515596	None	**P** (PVS1, PS1, PM2, PM3)	
			simplex AEF27	2:26,700,078:G > A	c.2485C > T: p.(Gln829Ter)	—	Stop_gained	Hetero	P/P	rs80356593	0.0172	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss**
				2:26,698,850:CGGCAAAGAGGCTGCGGGC > TGCGCTCGGAG	c.2905_2923delinsCTCCGAGCGCA: p.(Ala969LeufsTer30)	—	Frameshift	Hetero	P/P	rs397515596	None	**P** (PVS1, PS1, PM2, PM3)	
			multiplex NASF12	2:26,690,232:C > A	c.4227 + 1G > T	—	Splicing	Homo	P/LP-P	rs397515601	0.0008	**P** (PS1, PM1, PM2, PM3, PP1, PP3)	**Causative of hearing loss**
			simplex NAEF22	2:26,690,232:C > A	c.4227 + 1G > T	—	Splicing	Hetero	P/LP-P	rs397515601	0.0008	**P** (PS1, PM3, PM2, PP1, PP3)	**Causative of hearing loss**
				2:26698850CGGCAAAGAGGCTGCGGGC > TGCGCTCGGAG	c.2905_2923delinsCTCCGAGCGCA: p.(Ala969LeufsTer30)	—	Frameshift	Hetero	P/P	rs397515596	None	**P** (PVS1, PS1, PM2, PM3, PP1)	
			simplex E552	2:26,703,856:G > -	c.1601del	—	Frameshift	Hetero	n.l./P	rs397515583	None	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss**
				2:26,698,850:CGGCAAAGAGGCTGCGGGC > TGCGCTCGGAG	c.2905_2923delinsCTCCGAGCGCA: p.(Ala969LeufsTer30)	—	Frameshift	Hetero	P/P	rs397515596	None	**P** (PVS1, PS1, PM2, PM3, PP1)	

Variants were grouped by country and study

AN auditory neuropathy, *AR* presumptive autosomal recessive inheritance, *P* pathogenic, *LP* likely pathogenic, *VUS* variant of unknown significance, *B* benign., *n.l.* not listed, *Pop. Freq.* Population Frequency-based in gnomAD_exome

*present study (WES), ** present study (Sanger), *gnomAD, **ALFA, aTOPMED, #PAGE_STUDY

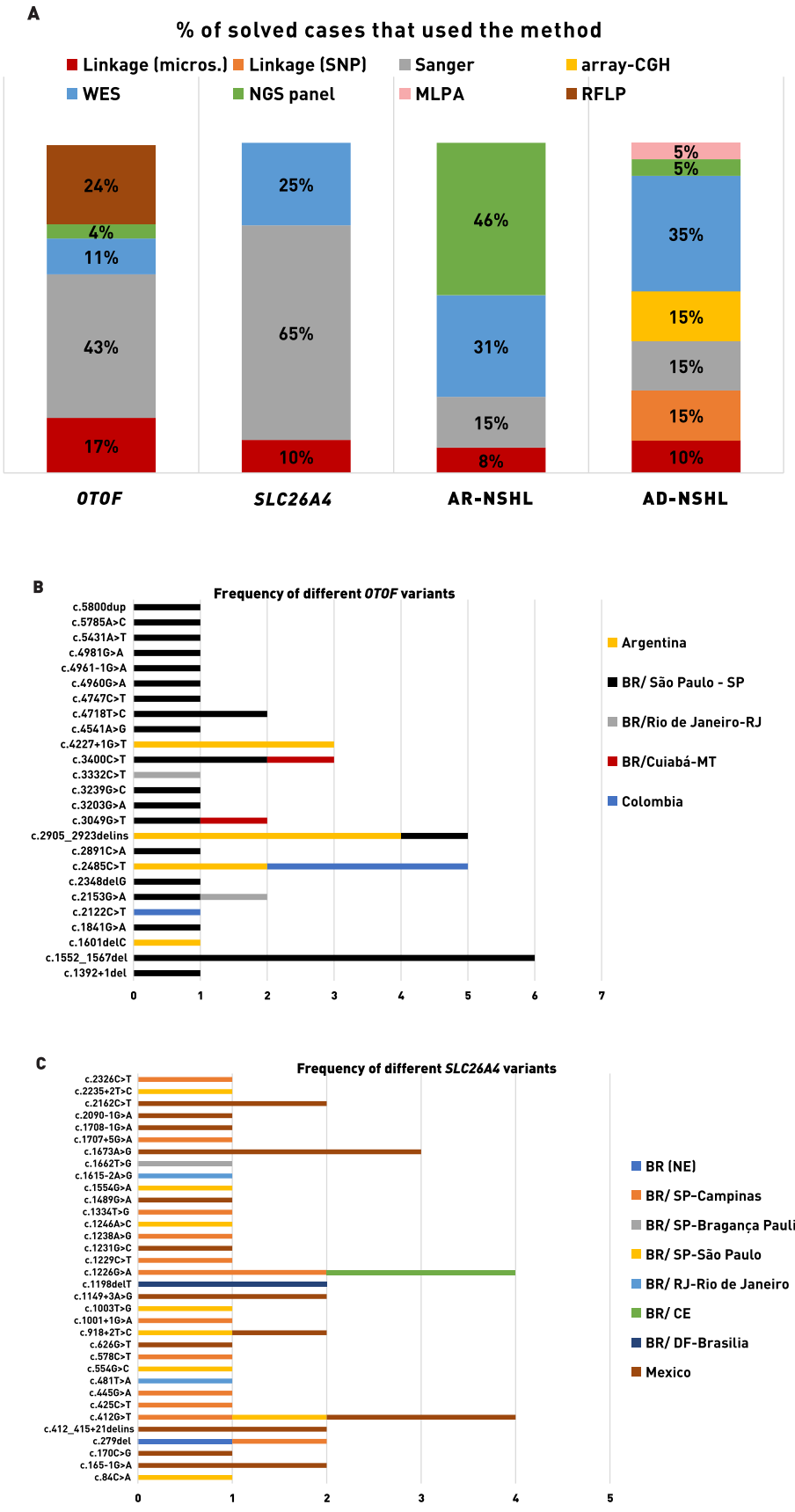


Fig. 5 **A** Estimates of the contribution of each method to the solution of cases in the screening of *SLC26A4*, *OTOF,* autosomal recessive non-syndromic hearing loss (AR-NSHL) and analysis of autosomal dominant non-syndromic hearing loss (AD-NSHL); **B** frequency of the different *OTOF* variants; **C** frequency of the different *SLC26A4* variants.

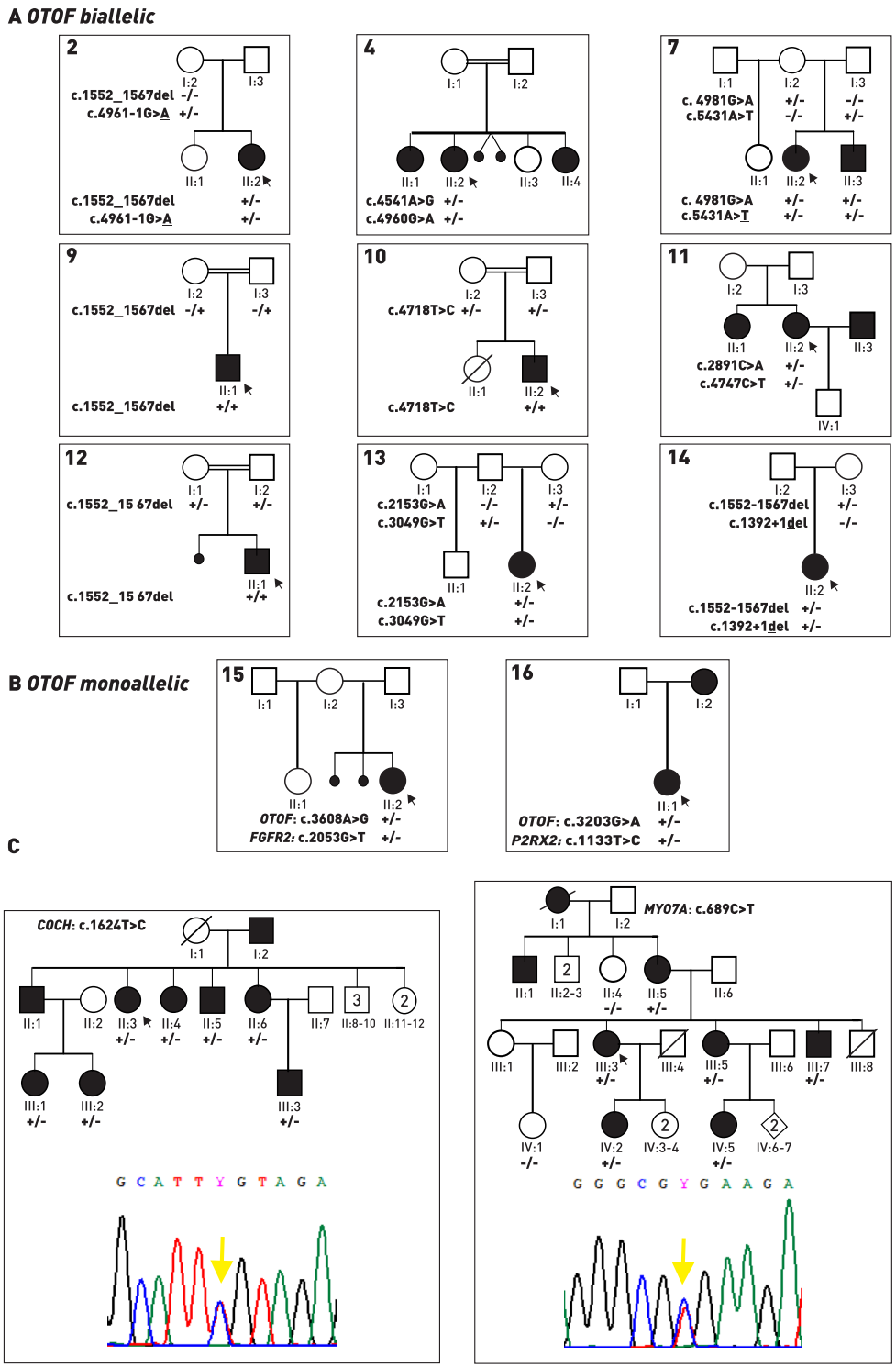


Fig. 6 Pedigrees showing the segregation of: **A** biallelic variants in *OTOF*; **B** monoallelic variants in *OTOF,* and the other candidate variants to explain the HL; **C** *COCH* and *MYO7A* variants

et al. 2009). Frequent HL variants had been previously excluded (c.35delG and c.167delT in *GJB2*, *GJB6* deletions and m.1555A > G). The p.(Gln829Ter) was not detected in any of the 342 probands, as mentioned above. Linkage analysis with microsatellites close to *OTOF* was conducted to prioritize probands to sequence all coding exons of the gene. In 11 cases, the linkage was compatible with *OTOF*, and among them, there was 1 documented auditory neuropathy (AN) case. Sequencing of all *OTOF* exons was carried out in 7 auditory neuropathy sporadic cases and the 11 cases putatively linked to *OTOF*. In eight cases, at least one likely pathogenic variant was detected, three biallelic and six monoallelic. Given the unsolved cases, we continued investigating the cases first reported by Romanos et al. (2009).

Table 2 shows the results obtained after the restudy of these cases, either with Sanger Sequencing or NGS, that allowed identifying additional pathogenic variants, thus solving three additional cases. Eight novel cases were also investigated for *OTOF* variants because of the clinical diagnosis of auditory neuropathy or AR inheritance, which revealed 12 additional variants. These variants were biallelic in five of the eight cases and were causative of the HL phenotype (Table 2). Considering the 16 subjects with likely pathogenic variants in *OTOF* (novel variants from the reexamined samples and additional subjects), we here report three novel variants: c.1392 + 1del, c.3049G > T: p.(Glu1017Ter) and c.4541A > G: p.(Asp1514Gly). The c.1392 + 1del variant is predicted to disrupt the donor splice site. The other two are loss of function variants, a frequent mutational mechanism in *OTOF*. The c.4541A > G: p.(Asp1514Gly) is predicted to be damaging/disease-causing by MutationTaster/Sift/Polyphen/Mutation Assessor/Provean and REVEL (score 0.923). In another recent study conducted in the city of São Paulo (Batissoco et al. 2021, this issue), two cases were selected because of the clinical diagnosis of auditory neuropathy. Biallelic causative variants in *OTOF* were found in the two cases screened, one case from the city of Cuiabá (Mato Grosso State from the MidWestern region of Brazil) and the other from Rio de Janeiro city (the Southeastern region).

Taking together, among all the pathogenic/likely pathogenic or unknown significance variants ($N = 30$) found in the city of São Paulo (Romanos et al.2009; Batissoco et al. 2021 this issue, and present study), there were remarkable cases of recurrent variants: six cases of the variant c.1552_1567del: p.(Arg518Thrfs*15) (frequency of 20%—6/30) reported only in Brazil, suggesting a common ancestor; two cases from São Paulo (6.7%-2/30) had the variant c.3049C > A: p.(Glu1017Ter), never reported before; the variant c.3400C > T: p.(Arg1134Ter) found in two patients in São Paulo, was previously reported twice in Italian patients (Santarelli et al. 2015; Rodríguez-Ballesteros et al. 2008); and the variant c.2153G > A: p.(Trp718Ter), also seen in two cases, was reported once in ClinVar and Deafness Variation Database (without reference to a publication). The presence of a recurrent "Italian" variant in the State of São Paulo is explained by the significant Italian contribution to the gene pool of Brazilians living in the state of São Paulo (and also in Southern states of the country), in comparison to other Brazilian regions, such as Northern and Northeastern, where Italian ancestry is less relevant. While considering all studies from Latin America, the c.2485 c.2905_2923delins variant stands out as a recurrent variant, detected in Argentina and Brazil as c.2485C > T: p.(Gln829Ter) in Colombia and Argentina (Fig. 5B).

In conclusion, *OTOF* variants were confirmed to be important as causing ARNSHL, especially in samples in which auditory neuropathy was the criterion for selecting subjects. Furthermore, the studies performed in Latin America contributed to the description of novel variants and, unexpectedly, p.(Gln829Ter) was not found very prominent despite the relevant Spanish contribution to the colonization of many countries.

SLC26A4 contribution to hearing loss in Latin American populations

Pendred syndrome (PDS) and Non-syndromic Enlarged Vestibular Aqueduct (DFNB4) represent part of the phenotypic spectrum resulting from pathogenic variants in *SLC26A4*, characterized by sensorineural hearing loss, usually of prelingual/perilingual onset, vestibular dysfunction, and temporal bone abnormalities, such as bilateral enlarged vestibular aqueduct with or without cochlear hypoplasia. Besides, PDS patients usually develop euthyroid goiter with a positive perchlorate test, later than the hearing and balance dysfunctions. Thus, some cases of PDS could be included in non-syndromic HL cohorts since there are patients with variants in *SLC26A4* who do not exhibit the clinical signs of Pendred syndrome or present the thyroid-related phenotypes later in life. Besides, intrafamilial phenotypic variation is possible, with individuals in the same pedigree presenting or not syndromic features. That is the reason why articles describing patients or families with pathogenic variants in *SLC26A4* were included in this review, regardless of the phenotypic classification (syndromic/non-syndromic).

At least 50% of PDS/DFNB4 cases are attributed to biallelic *SLC26A4* pathogenic variants or double heterozygosity with one variant in *SLC26A4* and the other in *FOXI1* or *KCNJ10* (Smith et al. 2020). Furthermore, many estimates account that those pathogenic alleles in *SLC26A4* might be the second most common cause of hereditary hearing loss after Connexin 26 (*GJB2*) mutations (Hilgert et al. 2009).

In many healthcare centers in Latin America, imaging exams are not routinely done to investigate hearing loss because of their elevated cost or lack of equipment. In addition, the goiter may not always manifest or may have a later onset. Therefore, the selection criteria of patients for *SLC26A4* studies were broad and heterogeneous in investigations that aimed at establishing its contribution among prelingual or autosomal recessive cases of HL.

Table 3 summarizes data about the pathogenic, likely pathogenic, and variants of unknown significance reported to date in Latin America in *SLC26A4*. Figure 5A illustrates the methods used to identify these variants. Figure 5C represents the frequency of the different variants among the countries.

Through linkage analysis and sequencing, Kopp et al. (1999) investigated a highly inbred pedigree from

Table 3 *SLC26A4* variants described in Latin America

Country/City or (Region)	Reference: Study design	Methods	Subjects	Genomic position (GRch37)	Updated variant description HGVS (*SLC26A4*, NM_000441)	Consequence	Copies	ClinVar/DVD	dbSNP	Pop. Freq. (%)	ACMG criteria and classification	ACMG criteria reanalysis
Brazil (NE)	Kopp et al (1999): 41 w/ features of PS, 3 homozygous w/*SLC26A4* **P** variant. Remaining presenting w/ deafness and/or goiter w/o molecular diagnosis	Linkage + Sanger	Multiplex inbed pedigree	7:107,303,855:T > -	c.279del: p.(Ser93ArgfsTer4)	Frameshift	Homo	P-LP/n.l.	rs786204421	0.0004	**P** (PVS1, PM2, PM3, PP1)	**Causative of hearing loss**
Brazil/São Paulo (SE)	Nonose et al. (2018): Sanger seq. in 2 groups: G1—16 w/ AR-NS-HL (68 tested) consistent w/linkage to *SLC26A4*; G2—15 w/ suspected PS and/or EVA or other cochlear-vestibular malformations	Linkage + Sanger	Mutliplex 6 (G1)	7:107,329,499:T > G	c.1003 T > G: p.(Phe335Val)	Missense	Hetero	CI /LP	rs111033212	None	**LP** (PM1, PM2, PM3, PP1, PP3)	**Causative of hearing loss**
				7:107,338,496:G > A	*c.1554G > A: p.(Trp518Ter)*	Stop gained	Hetero	P/n.l.	rs727503428	None	**P** (PVS1, PM2, PP1)	
			Multiplex 24 (G1)	7:107,302,170:C > A	c.84C > A: p.(Ser28Arg)	Missense	Hetero	P-LP/P	rs539699299	0.0014*	**P** (PS1, PM2, PM3, PP1, PP3)	**Causative of hearing loss**
				7:107,350,646:T > C	c.2235 + 2 T > C	Splicing	Hetero	LP/LP	rs1554362815	None	**LP** (PM2, PM3, PP1, PP3)	
			Simplex 71 (G2)	7:107,330,665:A > C	c.1246A > C: p.(Thr416Pro)	Missense	Hetero	P/P	rs28939086	0.0214*	**VUS** (PM2, PP3)	Inconclusive (monoallelic)
			Simplex 83 (G2)	7:107,323,801:T > C	c.918 + 2 T > C	Splicing	Hetero	P-LP/P	rs912147281	0.0008	**VUS** (PM2, PP3)	Inconclusive (monoallelic)
Brazil/Brasilia (MW)	Lofrano-Porto et al. (2008): 6 siblings w/ HL, EVA and goiter/thyroid phenotype	Sanger	Multiplex consang	7:107,330,616:TT > T	*c.1198del: p.(Cys400ValfsTer32)*	Frameshift	Homo	P-LP/n.l.	rs397516413	0.0028	**P** (PVS1, PS1, PM2, PM3, PP1)	**Causative of hearing loss**

Table 3 (continued)

Country/City or (Region)	Reference: Study design	Methods	Subjects	Genomic position (GRch37)	Updated variant description HGVS (*SLC26A4*, NM_000441)	Consequence	Copies	ClinVar/DVD	dbSNP	Pop. Freq. (%)	ACMG criteria and classification	ACMG criteria reanalysis
Brazil/Campinas (SE)	de Moraes et al. (2013)*: 23 cases of NS-HL w/EVA	Sanger	2	7:107,330,648:C > T	c.1229C > T: p.(Thr410Met)	Missense	Hetero	P/P	rs111033220	0.0043*	**LP** (PS1, PM2, PM3, PP3)	**Causative of hearing loss**
				7:107,340,625:G > A	c.1707 + 5G > A	Splicing	Hetero	P/P	rs192366176	0.0008	**LP** (PS1, PM2, PM3, PP3)	
			6	7:107,312,690:G > T	c.412G > T: p.(Val138Phe)	Missense	Hetero	P/LP	rs111033199	0.0175	**LP** (PS1, PM2, PM3, PP3)	**Causative of hearing loss**
				7:107,330,657:A > G	c.1238A > G: p.(Gln413Arg)	Missense	Hetero	P-LP/P	rs142498437	0.0008	**LP** (PS1, PM2, PM3, PP3)	
			21	7:107,314,618:C > T	c.425C > T: p.(Pro142Leu)	Missense	Hetero	n.l./P	rs1790948425	0.000**	**LP** (PS1, PM2, PM3, PP3)	**Causative of hearing loss**
				7:107,303,855:GT > G	c.279del: p.(Ser93ArgfsTer4)	Frameshift	Hetero	P/n.l.	rs786204421	0.0004	**P** (PVS1, PM2, PM3, PP3)	
			22	7:107,330,645:G > A	c.1226G > A: p.(Arg409His)	Missense	Homo	P/P	rs111033305	0.006[a]	**LP** (PS1, PM2, PM3, PP3)	**Causative of hearing loss**
			23	7:107,334,918:T > G	c.1334 T > G: p.(Leu445Trp)	Missense	Hetero	P/P	rs111033307	0.01	**LP** (PS1, PM2, PM3, PP3)	**Causative of hearing loss**
				7:107,323,983:G > A	c.1001 + 1G > A	Splicing	Hetero	P/P	rs80338849	0.0298**	**LP** (PS1, PM2, PM3, PP3)	
			15	7:107,355,874:C > T	c.2326C > T: p.(Arg776Cys)	Missense	Hetero	CI/LP	rs111033255	0.18	**LP** (PS1, PM2, PM3, PP3)	Monoallelic (inconclusive)
			16	7:107,314,638:G > A	c.445G > A: p.(Gly149Arg)	Missense	Hetero	VUS/P	rs761210511	0.0004	**LP** (PS1, PM2, PP3)	Monoallelic (inconclusive)
			18	7:107,314,771:C > T	c.578C > T: p.(Thr193Ile)	Missense	Hetero	P-LP/P	rs111033348	0.002	**LP** (PS1, PM2, PP3)	Monoallelic (inconclusive)

Table 3 (continued)

Country/City or (Region)	Reference: Study design	Methods	Subjects	Genomic position (GRch37)	Updated variant description HGVS (*SLC26A4*, NM_000441)	Consequence	Copies	ClinVar/DVD	dbSNP	Pop. Freq. (%)	ACMG criteria and classification	ACMG criteria reanalysis
Brazil/São Paulo (SE)	Batissoco et al. (2021): From 28 cases w/ cochleo-vestibular malformations, 15 w/no other clinical features were screened for *SLC26A4*	Sanger	S1	7:107,312,690:G > T	c.412G > T: p.(Val138Phe)	Missense	Hetero	LP/P	rs111033199	0.0175	**P** (PS1, PM2, PM3, PP1, PP3)	**Causative of hearing loss**
				7:107,314,747:G > C	c.554G > C: p (Arg185Thr)	Missense	Hetero	LP/P	rs542620119	0.0091	**P** (PS1, PM2, PM3, PP1, PP3)	
Brazil/Rio de Janeiro (SE)			S2	7:107,340,526:A > G	c.1615-2A > G	Splicing	Hetero	P/P	rs758823761	0.0004	**LP** (PM1, PM2, PM3, PP1, PP3)	**Causative of hearing loss**
				7:107,314,674:T > A	c.481 T > A: p.(Phe161Ile)	Missense	Hetero	VUS/P	rs1481765326	0.0008	**LP** (PM1, PM2, PM3, PP1, PP3)	
Brazil/Bragança Paulista (SE)			S9	7:107,340,575:T > G	c.1662 T > G: p.(Ile554Met)	Missense	Hetero	n.l./n.l.	rs908303704	none	**VUS** (PP3, PM1, PM2) unknown relation w/ the phenotype	Inconclusive (monoallelic)
Brazil/Ceará countryside (NE)			S10	7:107,330,645:G > A	c.1226G > A: p.(Arg409His)	Missense	Homo	P/P	rs111033305	0.006[a]	**LP** (PS1, PM2, PP3, PP1)	**Causative of hearing loss**
Mexico	Gonzalez-Trevino et al. 2001: PR SNHL w/ small goiter at birth and Mondini + EVA	Sanger	Simplex Family 1	7:107312690_107312714:delinsTGACA	c.412_415 + 21delinsTGACA	Frameshift	Homo	n.l./n.l.	n.l.	n.l.	**P** (PVS1, PM2, PP1)	**causative of hearing loss**
Mexico	Gonzalez-Trevino et al. 2001: 2 siblings w/ PR SNHL, goiter/thyroid phenotype and Mondini + EVA	Sanger	Multiplex Family 2	7:107,303,740:G > A	c.165-1G > A	Splicing	Hetero	P-LP/P	rs759792660	0.00048	**LP** (PM1, PM2, PM3, PP1, PP3)	**causative of hearing loss**
				7:107,330,650:G > C	c.1231G > C: p.(Val411Pro)	Missense	Hetero	LP/LP	rs1293971731	0.0008	**LP** (PM1, PM2, PM3, PP1, PP3)	
Mexico	Gonzalez-Trevino et al. 2001: PR SNHL w/ goiter noted at 18yo and Mondini + EVA	Sanger	Simplex Family 3	7:107,312,690:G > T	c.412G > T: p.(Val138Phe)	Missense	Homo	P/P	rs111033199	0.00175	**P** (PS1, PM2, PM3, PP1, PP3)	**causative of hearing loss**
Mexico	Cengiz et al. (2017): bilateral Mondini	WES	Multiplex 2215	7:107,340,586:A > G	c.1673A > G: p.(Asn558Ser)	Missense	Homo	VUS/P	rs766206507	0.0044	**LP** (PM1, PM2, PM3, PP1, PP3)	**causative of hearing loss**

Table 3 (continued)

Country/ City or (Region)	Reference: Study design	Methods	Subjects	Genomic position (GRch37)	Updated variant description HGVS (*SLC26A4*, NM_000441)	Consequence	Copies	ClinVar/ DVD	dbSNP	Pop. Freq. (%)	ACMG criteria and classification	ACMG criteria reanalysis
Mexico	Cengiz et al. (2017): bilateral EVA	Sanger	Simplex 1169	7:107,340,586:A > G	c.1673A > G: p.(Asn558Ser)	Missense	Hetero	VUS/P	rs766206507	0.0044	**P** (PS1, PM2, PM3, PP1, PP3)	**causative of hearing loss**
				7:107,323,801:T > C	c.918 + 2 T > C	Splicing	Hetero	P-LP/P	rs912147281	0.0008	**P**(PS1, PM2, PM3, PP1, PP3)	
Mexico	Cengiz et al. (2017): bilateral EVA		Mutliplex 1393	7:107,303,746:C > G	c.170C > G: p.(Ser57Ter)	Stop gained	Hetero	P/LP	rs111033200	0.0012	**P** (PVS1, PS1, PM2, PM3)	**causative of hearing loss**
				7:107,341,545:G > A	c.1708-1G > A	Splicing	Hetero	LP/P	rs759414956	0.0004	**P** (PS1, PM2, PM3, PP1, PP3)	
Mexico	Cengiz et al. (2017): bilateral EVA	WES	Simplex 2211	7:107,303,740:G > A	c.165-1G > A	Splicing	Hetero	P-LP/P	rs759792660	0.00048	**P** (PS1, PM2, PM3, PP1, PP3)	**causative of hearing loss**
				7:107,315,415:G > T	c.626G > T: p.(Gly209Val)	Missense	Hetero	P/P	rs111033303	0.0302	**P**(PS1, PM2, PM3, PP1, PP3)	
Mexico	Cengiz et al. (2017): bilateral EVA	WES	Multiplex 2214	7:107,336,429:G > A	c.1489G > A: p.(Gly497Ser)	Missense	Hetero	P-LP/P	rs111033308	0.002	**P** (PS1, PM2, PM3, PP1, PP3)	**causative of hearing loss**
				7:107,350,498:G > A	c.2090-1G > A	Splicing	Hetero	LP/LP	rs1455597424	0.0008	**P** (PS1, PM2, PM3, PP1, PP3)	
Mexico	Cengiz et al. (2017): bilateral EVA	Sanger	Simplex 1180	7:107,329,648:A > G	c.1149 + 3A > G	Splicing	Homo	P/P	rs111033314	0.0024	**P** (PS1, PM2, PP1, PP3)	**causative of hearing loss**
Mexico	Cengiz et al. (2017): bilateral EVA	WES	Multiplex 1318	7:107,350,571:C > T	c.2162C > T: p.(Thr721Met)	Missense	Homo	P/P	rs121908363	0.0052	**P** (PS1, PM2, PP1, PP3)	**causative of hearing loss**

Variant position and nomenclature according to Deafness Variation Database. The variant c.1554G > A: p.(Trp518Ter) was described as c.1553G > A in the publication of Nonose et al. (2018). *Variant c.1198delT: p.(Cys400ValfsTer32) was described as c.1197del in the publication* Lofrano-Porto et al. (2008)

Variants were grouped by country and study

PS = Pendred Syndrome, PR = prelingual, P pathogenic, *LP* likely pathogenic, *VUS* variant of unknown significance. *n.l.* not listed, *Pop. Freq.* population frequency-based in gnomAD_exome

*de Moraes et al. did not report if the cases were simplex or multiplex

*gnomAD, **ALFA, aTOPMED, #PAGE_STUDY

Northeastern Brazil, with the affected individuals showing features of Pendred syndrome. They identified three family members affected by deafness, positive perchlorate test, and goiter, who were homozygous for NM_000441.2:c.279del: p.(Ser93ArgfsTer4). Surprisingly, there were 19 patients with HL and/or goiter who were monoallelic or did not carry the *SLC26A4* variant. The goiters were found to be due to iodine deficiency, and HL was probably due to another autosomal recessive gene. Investigation of another consanguineous Brazilian family in which Pendred syndrome was segregating revealed wide variability in the clinical presentation. Sanger sequencing identified the c.1198delT:p.(Cys400ValfsTer32) homozygous variant in all three deaf siblings (Lofrano-Porto et al. 2008; Table 3).

A total of 31 unrelated Brazilian patients from São Paulo city, Brazil, were screened for variants in the *SLC26A4* gene based on 2 selection criteria: 16 index cases with presumptive autosomal recessive inheritance, with microsatellite haplotype segregation compatible with DFNB4, selected from a collection of 68 pedigrees; and 15 probands who were suspected of presenting Pendred syndrome, because of the presence of hearing loss associated with thyroid dysfunction, or because hearing loss was associated with EVA or other inner ear malformation (Nonose et al. 2018). Among the autosomal recessive cases, biallelic pathogenic variants (Table 3) were detected in two cases, 12.5% (2/16 or 2/68 from the total of pedigrees). In the group with suspected Pendred syndrome, two monoallelic cases were detected, 13.3% (2/15). Exome sequencing and MLPA failed to find a second causative variant. Another study, also performed in São Paulo (Batissoco et al. 2021, this issue), aimed to determine the frequency of *SLC26A4* variants in 15 cases of HL associated with inner ear malformations with no additional clinical features. Three biallelic (20%) and one monoallelic case (7%) were found.

Among 23 unrelated Brazilian patients with NSHL and EVA from Campinas, screening of the *SLC26A4* gene revealed biallelic pathogenic variants in 5 (21.7–5/23), and 3 were monoallelic (Table 3; de Moraes et al. 2013). The contribution of *SLC26A4* to prelingual NSHL was also estimated by applying the High-Resolution Melting technique to screen 88 samples from Campinas city, followed by Sanger sequencing, but no causative variants were identified (Carvalho et al. 2018). However, two monoallelic cases were identified with novel missense variants, NM_000441.2:c.760A > G: p.Ile254Val and NM_000441.2:c.1146C > G: p.Asn382Lys.

Four Mexican patients from three unrelated families presenting with sensorineural deafness, Mondini malformations of the cochlea, an enlarged vestibular aqueduct, goiter, and a positive perchlorate test, were investigated through linkage analysis and sequencing. Biallelic pathogenic variants (Table 3) were found in all three families (Gonzalez-Trevino et al. 2001). In a multicenter study, 7 probands among 11 (63%), from Mexico, with non-syndromic sensorineural HL and inner ear anomalies were found to have biallelic pathogenic variants in *SLC26A4*. Bademci et al. (2016) used exome NGS to investigate the molecular causes of non-syndromic HL in two probands from Mexico, two from Ecuador, and one from Puerto Rico. One Mexican patient was biallelic regarding variants in *SLC26A4*.

The most frequent variants, described in Table 3 and Fig. 5C, were c.1226G > A: p.(Arg409His) and c.412G > T: p.(Val138Phe)—present in four alleles each, followed by c.1673A > G: p.(Asn558Ser) present in three alleles, and seven other variants that were present in two alleles each. While c.1226G > A: p.(Arg409His) was found only in Brazil, its highest population frequency was described among Americans (Latino), according to GnomAD, but it has also been reported to be frequent in Iran and Turkey, probably sharing a common founder in those regions (Bademci et al. 2006). It is possible that c.1226G > A: p.(Arg409His) was inherited from a common ancestor in Eastern countries, and Latin America, given the diversity of Latin American parental populations and the genetic contributions it received after the nineteenth century. The c.412G > T: p.(Val138Phe) variant was detected in two cohorts, in São Paulo State as well as in Mexico, and it was recurrent in a comprehensive study of North American patients (Sloan-Heggen et al. 2016). It was also frequent in Germany, Czech Republic, and Denmark (Tsukada et al. 2015a).

Overall, *SLC26A4* variants were shown to have a relevant contribution to the etiology of HL among a specific subgroup of non-syndromic patients, mainly selected because they are affected by inner ear malformations. However, its contribution to the etiology of hearing loss among all non-syndromic cases is hard to estimate, given that most studies focused on selected samples because of DFNB4 or PS-related phenotypes.

Latin American families reveal impressive genetic heterogeneity in autosomal recessive hearing loss, even within pedigrees

Some Latin American cities are densely populated, with sizes comparable to the most populous metropolis in the world. For instance, the city of São Paulo in Brazil has an estimated 12 million people, and Mexico City has nearly 9 million people. In these modern and populated cities, there is an overall trend of reducing family sizes and reducing consanguineous unions. For instance, Brazil had a fertility rate estimated to be 2.4 near IBGE 2020, but in 2020 it changed to 1.7 (IBGE, 2020). Although consanguineous unions are nowadays rare in big cities, there are still thousands of minor cities, villages, or rural properties spread over Latin America in which inbreeding is still highly significant. For instance, Weller et al. (2012) and Otto et al. (2020) dealt with the types and frequency of consanguineous unions in Northeastern Brazil. Although the level of inbreeding is reduced compared to other populations,

Table 4 Variants in autosomal recessive (AR) genes detected in Latin America, segregating with non-syndromic bilateral sensorineural HL

Country (Region)/ City or State	Reference: Study design	Methods	Genomic position (GRch37)	Gene/ Locus	Updated variant description HGVS	Consequence	Copies	Clin Var/ DVD	dbSNP	No of families/ affected individuals
Brazil (NE) Piauí State	Lezirovitz et al. (2008): PR severe to profound NS-SNHL, inbred pedigree, 5 w/o these *MYO15A variants*	Linkage Analysis + Sanger	17:18,082,163:CA > C	*MYO15A*/ DFNB3	NM_016239.4: c.10573del: p.(Ser3525AlafsTer29)	Frameshift	Homo	P/P	rs1270302810	1/15
			17:18,082,163:CA > C	MYO15A/DFNB3	NM_016239.4: c.10573delA	Frameshift	Hetero	P/P	rs1270302810	1/5
			17:18,070,910:CCTGA > C		NM_016239.4:c.9958_9961del	Frameshift	Hetero	P/P	rs1567664131	
			17:18,082,163:CA > C	*MYO15A*/ DFNB3	NM_016239.4: c.10573del: p.(Ser3525AlafsTer29)	Frameshift	Hetero	P/P	rs1270302810	Monoallelic (1)
Brazil (SE) São Paulo State	Dias et al. (2019): PP HL, inbred pedigree	NGS panel 99 genes	19:45,207,341:C > T	*CEACAM16*	NM_001039213.4:c.436C > T:p.(Arg146Ter)	Stop gained	Homo	P	rs1435499034	1/3
Mexico	Bademci et al (2016): NS-SNHL w/ at least two affected, presumptive AR	WES	10:73,466,659:G > A	*CDH23*/ DFNB12	c.2959G > A: p.(Asp987Asn)	Missense	Hetero	P/VUS	rs770665588	1/1
			10:73,490,274:C > T		c.3628C > T: p.(Gln1210Ter)	Stop gained	Hetero	P/P	rs397517326	
Ecuador		WES	9:75,431,081:T > A	*TMC1*/ DFNB7/11	NM_138691.2:c.1718 T > A: p.(Ile573Asn)	Missense	Hetero	LP	—	1/1
			9:75,445,365:AG > A		NM_138691.2:c.2130-1del	Splicing	Hetero	LP	—	
Puerto Rico		WES	17:18,053,754:GC > G	*MYO15A*/ DFNB3	NM_016239.4:c.7226del: p.(Pro2409GlnfsTer8)	Frameshift	Hetero	P/P	rs759151127	1/1
			17:18,066,565:G > A		c.9620G > A: p.(Arg3207His)	Missense	Hetero	P/CI	rs199621031	

Table 4 (continued)

Country (Region)/ City or State	Reference: Study design	Methods	Genomic position (GRch37)	Gene/ Locus	Updated variant description HGVS	Consequence	Copies	Clin Var/ DVD	dbSNP	No of families/ affected individuals
Brazil (NE) Monte Santo-BA	Manzoli et al. (2016): 15 unrelated probands, w/ NS-SNHLNS-SNHL putative AR from Monte Santo, Bahia (BA) (Northeast), 1 from Salvador (BA) (Northeast) and 3 from Porto Alegre(RS) (South)	NGS panel180 genes	17:18,034,837:G > A	*MYO15A*/ DFNB3	NM_016239.4:c.4198G > A: p.(Val1400Met)	Missense	Homo	P/P	rs749136456	6/?
Brazil (NE) Monte Santo-BA		NGS panel180 genes	17:18,034,837:G > A	*MYO15A*/ DFNB3	NM_016239.4:c.4198G > A: p.(Val1400Met)	Missense	Hetero	P/P	rs749136456	2
			17:18,058,027:C > T		NM_016239.4:c.8182C > T: p.(Arg2728Cys)	Missense	Hetero	P	rs758464431	
Brazil (S) Porto Alegre-RS		NGS panel180 genes	17:18,063,264:G > T	*MYO15A*/ DFNB3	NM_016239.4:c.9319G > T:p.(Glu3107Ter)	Stop gained	Homo	P	—	?
Brazil (S) Porto Alegre-RS		NGS panel180 genes	17:18,054,586:C > T	*MYO15A*/ DFNB3	NM_016239.4:c.7636C > T:p.(Gln2546Ter)	Stop gained	Hetero	P	rs765936685	?
			17:18,063,264:G > T		NM_016239.4:c.9319G > T:p.(Glu3107Ter)	Stop gained	Hetero	P	—	?
Brazil (S) Porto Alegre-RS		NGS panel180 genes	21:37,833,703:G > T	*CLDN14*/ DFNB29	NM_001146077.1:c.291C > Ap.(Cys97Ter)	Stop Gained	Homo	P	rs767108790	1

Table 4 (continued)

Country (Region)/ City or State	Reference: Study design	Methods	Genomic position (GRch37)	Gene/ Locus	Updated variant description HGVS	Consequence	Copies	Clin Var/ DVD	dbSNP	No of families/ affected individuals
Brazil (SE) São Paulo State	Batissoco et al. (2021): 21 cases selected for NGS among 542 cases, 31 cases selected for *TMPRSS3* screening through Sanger because PP HL presumptive AR	NGS panel ~ 100 genes	17:18,023,729:C > T	*MYO15A/ DFNB3*	1615C > T: p.(Gln539Ter)	Stop gained	Hetero	P/P	rs1597752877	1/1
			17:18,025,637:C > CA		c.3524_3525insA:p.(Ser1176ValfsTer14)	Frameshift	Hetero	P/P	rs766187994	
Brazil (S) Paraná State		WES	21:43,795,896:C > T	*TMPRSS3/* DFNB8/10	c.1276G > A:p.(Ala426Thr)	Missense	Hetero	P/LP	rs56264519	1/3
			21:43,802,210:C > T		c.916G > A:p.(Ala306Thr)	Missense	Hetero	P/P	rs181949335	
Brazil (SE) Rio de Janeiro State		Sanger	21:43,808,612:C > T	*TMPRSS3/* DFNB8/10	c.346G > A:p.(Val116Met)	Missense	Hetero	LP/LP	rs200090033	1/1
			21:43,808,545:G > T		c.413C > A: p.(Ala138Glu)	Missense	Hetero	P/LP	rs147231991	

PP postlingual progressive, P pathogenic, *LP* likely pathogenic, *VUS* variant of unknown significance, *CI* conflicting interpretations of pathogenicity

for instance, those from Pakistan, one occasionally finds in Latin America highly inbred families, which have been helpful to the identification of novel genes and variants related to HL, which are shown in Table 4. The methods used to unravel these variants are listed in Fig. 5A. Surprisingly, the assumption that all cases of genetic disease in a large inbred family are due to one single pathogenic variant in homozygosis has been proved wrong in some cases. This unexpected genetic heterogeneity was reported by Lezirovitz et al. (2008) in a large inbred Brazilian pedigree with 26 subjects affected by prelingual deafness, with autosomal recessive inheritance. Instead of one expected homozygous mutation in a single gene, indicated as the *MYO15A* gene (DFNB3 locus) by linkage studies, two different pathogenic variants and possibly a third undetected one were found in the same pedigree within this gene. Among the 26 affected subjects, 15 were homozygous with NM_016239.4: c.10573delA: p.(Ser3525AlafsTer29) [5 were compound heterozygotes with a second variant NM_016239.4: c.9958_9961del: p.(Asp3320ThrfsTer2), and 1 inherited only a single c.10573delA, without a second variant identified. There might be other deafness loci segregating to explain the condition in some of the subjects in the same pedigree, whose deafness was not due to *MYO15A* mutations. Another example of genetic heterogeneity within one pedigree was presented in the reports of Lezirovitz et al. (2006) and Dias et al. (2019), in which the same extended genealogy was investigated. In the work of Lezirovitz et al. (2006), it was identified that some individuals, born from a union reported as nonconsanguineous (but in which haplotyping showed evidence of a common ancestor), presented with oculocutaneous albinism due to a homozygous variant in the *MATP* gene (OCA4 locus) and some presented prelingual deafness due to c.35delG in *GJB2* in homozygosis. In a second sibship from the same inbred pedigree, Dias et al. (2019), using massive parallel sequencing, demonstrated that hearing loss was due to the variant c.436C > T:p.(Arg146Ter) in homozygosis in the *CEACAM16* gene, segregating with postlingual progressive hearing loss with autosomal recessive inheritance. The latest report was significant to confirm the previous findings of Booth et al. (2018), who associated, for the first time, *CEACAM16* to autosomal recessive hearing loss.

Massive parallel sequencing of exome or targeted sequencing of hearing loss-related genes has recently revealed a wide repertoire of novel variants, some never described in other continents. Bademci et al. (2016) used exome sequencing to investigate a multiethnic cohort of hearing-impaired subjects from pedigrees with presumptive autosomal recessive hearing loss. One of the two patients from Mexico was biallelic for *CDH23*, c.2959G > A: p.(Asp987Asn)/c.3628C > T: p.(Gln121Ter), one of two from Ecuador was a biallelic for *TMC1*, c.1718 T > A: p.(Ile573Asn)/c.2130-1delG, and the patient from Puerto Rico had biallelic variants in *MYO15A,* c.7226delC: p.(Pro2409GlnfsTer8)/c.9620G > A: p.(Arg3207His). Near half of the variants identified in the study were novel. In the study of Manzoli et al. (2016), targeted sequencing of 180 hearing loss genes was performed in 19 probands of Brazilian families, most from the Northeastern region. The authors identified pathogenic variants in *MYO15A* (ten families) and *CLDN14* (one family). It was remarkable that one specific variant, p.(Val1400Met) in *MYO15A*, was found in eight families from one city, and haplotype analysis was consistent with one single origin for the variant. In another report, *MYO15A* variants, c.1615C > T: p.(Gln539Ter)/c.3524_3525insA:p.(Ser1176ValfsTer14), both already reported as pathogenic and identified through an NGS panel of ~ 100 HL genes in a sporadic case of bilateral prelingual HL (Batissoco et al. 2021, this issue). Summing up, there are 15 reported families of AR-HL associated with *MYO15A* in Latin America, with 7 different pathogenic variants, and only 2 recurrent variants were found in inbred communities. Thus, it is likely that this gene represents an important cause of AR-HL in Brazil or Latin America, but its large size makes NGS mandatory, which is not yet affordable as a routine even in the richest cities of Latin American.

The *TMPRSS3* might also be a relevant cause of AR-HL in Brazil (Batissoco et al. 2021, this issue) since three siblings from the same family affected by postlingual progressive HL were found to be compound heterozygotes with two pathogenic variants using WES, c.1276G > A:p.(Ala426Thr)/c.916G > A:p.(Ala306Thr). These findings motivated screening of *TMPRSS3* using Sanger sequencing in a selected sample of 31 cases of postlingual progressive HL with presumptive autosomal recessive inheritance. One isolated case was detected as a compound heterozygote with two *TMPRSS3* pathogenic variants, c.346G > A:p.(Val116Met)/c.413C > A: p.(Ala138Glu).

These reports reinforce that founder effects may account for specificities in diversity and frequency of variants. Specific and regional distribution of variants influences the planning strategies for developing genetic testing routines in different populations.

Genetic analysis of autosomal dominant hearing loss pedigrees: novel candidate variants and genes revealed

Although there is a recent general trend for reducing children in sibships, it is still common to ascertain, in genetic counseling services, large multigenerational pedigrees with large offspring, with many individuals affected by genetic diseases exhibiting autosomal dominant inheritance. Ascertained in genetic services located in the greatest cities because of individuals who migrated, these large pedigrees frequently

Table 5 Variants in autosomal dominant (AD) genes detected in Latin America, segregating with non-syndromic bilateral sensorineural HL

Country/City	References	Study Design, HL Phenotype	Methods	Gene/Locus	Genomic location (GRch37)	Variant in DNA/Protein	Variation Databases	Consequence	No of families/affected individuals
Costa Rica	Leon et al. (1992), Lynch et al. (1997)	NS-SNHL, PP, Onset at ~ 10 years old, low-frequency,	Genomic Linkage analysis (micros.), Sanger seq., Transcript analysis of lymphoblastoid cell lines	*DIAPH1*/DFNA1	5:140,903,709:C > A	NM_005219.5: c.3661 + 1G > T	DVD/ClinVar: P	splice_donor site disruption	1/78
Brazil/São Paulo	Lezirovitz et al. (2012)	NS-SNHL, onset at early childhood to adolescence, non-progressive, severe-profound	Linkage analysis (micros.), Sanger seq., Transcript analysis of lymphoblastoid cell lines	*TECTA*/DFNA8/12	11:121,036,091:TAGTG > T	NM_005422.2: c.5383 + 5_5383 + 8del: p.(Ser1758Tyr/Gly1759_Asn1795del)	DVD: P, ClinVar: SCV001762976	splice_donor site disruption	1/9
Brazil/São Paulo	Dantas et al. (2014) (variant first reported by Lalwani et al. 2000)	NS-SNHL, PP, onset from 1st to 5th decade of life,	Genomic Linkage analysis (SNP), WES	*MYH9*/DFNA17	22:36,702,021:C > T	NM_002473.5: c2114G > A: p.(Arg705His)	DVD/ClinVar: P	Missense	1/10
Brazil/São Paulo	Freitas et al. (2014), Rosenberg et al. (2016)	NS-SNHL, PP. onset at adolescence, progressive	Oligonucleotide array-CGH	*POU4F3*/DFNA15	56 Kb-1137 Kb deletion in 5q32 NC_000005.9: g.(145678252_145702343)_(145758292_145815858)	Deletion of the whole gene	—	CNV	1/2
Brazil/São Paulo	Rosenberg et al. (2016)	50 cases of AR-NSHL and 50 cases of AD-NSHL studied, 2 CNV found	Oligonucleotide array-CGH	*EYA4*/DFNA10	6:133,517,648–133,693,108 (175 Kb)	Deletion of the whole gene	—	CNV	1/1

Table 5 (continued)

Country/City	References	Study Design, HL Phenotype	Methods	Gene/Locus	Genomic location (GRch37)	Variant in DNA/Protein	Variation Databases	Consequence	No of families/affected individuals
Brazil/São Paulo	Uehara et al. (2015), Rosenberg et al. (2016)	132 cases, 1 familial w/both a private duplication (0.7%) and a novel missense variant	Oligonucleotide array-CGH and customized MLPA, WES	*KCNQ4*/DFNA2A	1:41,284,345:A > T	NM_004700.4: c.701A > T: p.(His234Leu)	DVD: P, ClinVar: SCV001762977	Missense	1/3
				IMMP2L and *DOCK4* partial dup	7q31.1—7:110,942,457 to 111,401,476 bp; GRCh37/hg19		VUS	CNV	1/3
Brazil/São Paulo	Sampaio-Silva et al. (2018)	Four familial cases w/ AD-NSHL, 1 of which w/PP NS-SNHL, onset ~ 19 to 60 years ol	WES	*MYO6*/DFNA22	6:76,599,832:C > A	NM_004999: c.2717C > A: p.(Ser906Ter)	DVD/ClinVar: P	stop_gained	1/13
Brazil/São Paulo	Dantas et al. (2018)	2 familial cases of AD-NSHL, PP, all frequencies, mild-severe, av. onset ~ 30 years old	Linkage analysis, WES, SNP-array genotyping and Kinship analyses, Functional assays	*MYO3A*	10:26,414,513:T > G	NM_017433: c.2090 T > G: p.(Leu697Trp)	LOVD/ClinVar/ DVD: P	Missense	1/26 1/10
Brazil/São Paulo	Bueno et al. (2021)	101 AD-NSHL cases, 13 by NGS panel (1 positive) and 88 w/ Sanger (2 positive): av. onset ~ 30 years old PP, all frequencies	100 HL genes NGS panel, Sanger Seq						1/7 1/1 1/1

Table 5 (continued)

Country/City	References	Study Design, HL Phenotype	Methods	Gene/Locus	Genomic location (GRch37)	Variant in DNA/Protein	Variation Databases	Consequence	No of families/affected individuals
Brazil/São Paulo	Lezirovitz et al. (2009), Lezirovitz et al. (2020)	PP, av. onset ~ 18 years old, NS-SNHL	Genome Scan Linkage analysis (microsatellites), WES, array-CGH, MLPA, Gene Expression analysis	DFNA58—NC_000002.12:g.(68474704_68 475 209)_(68676657_68 679 298) dup [~ 200 Kb Genomic duplication in 2p: *PLEK*,*CNRIP1*, *PPP3R1*, *AC017083.3, LOC107985892, LOC102724389* and *LOC101927723/AC015969.1*]			European Variation Archive (EVA)	CNV	1/20
Brazil/São Paulo	Salazar-Silva et al. (2021)	NS-SNHL, bilateral and PP av. onset ~ 12 years old	Genomic Scan Linkage analysis (SNP microarray), WES, Sanger seq., Gene Expression analysis and Functional analysis	*NCOA3*	20:46,268,423:C > G	NM_181659: c.2810C > G: p.(Ser937Cys)	rs142951578, DVD: n.l./ ClinVar: SCV001762980	Missense	1/7
Brazil/São Paulo	present study (variant first reported by Di Leva et al. 2006)	NS-SNHL, PP. av. onset ~ 12 yo	WES	*MYO7A*/DFNA11	11:76,868,004:C > T	NM_000260.4: c.689C > T: p.(Ala230Val)	DVD: **P**/ClinVar: **P/LP**	Missense	1/6
Brazil/São Paulo	present study (variant first reported by Tsukada et al. 2015b)	NS-SNHL, pp, av. onset ~ 20 years old,	WES	*COCH*/DFNA9	14:31,358,968:T > C	NM_004086: c.1624 T > C: p.Cys542Arg	DVD: **P**; ClinVar: SCV001762979	Missense	1/7

Except for the variant in *DIAPH1* that was identified in a family from Costa Rica, the other studies reported Brazilian families. NS-SNHL = Non-Syndromic SensoriNeural Hearing Loss PP = postlingual progressive

have their ancestors in smaller cities or rural regions, where families are still large. Our laboratories in the city of São Paulo have ascertained an exciting collection of samples from extended families with autosomal dominant hearing loss, who were personally examined by us or were examined by collaborators in other regions of the country who provided us with samples for molecular studies. Some of these large pedigrees allowed linkage mapping studies with LOD score calculations, followed by investigating the segregation of novel variants to validate their pathogenicity. Massive parallel sequencing of the exome was crucial in many cases to find the causative genetic alterations, as shown in Fig. 5A, summarizing the methods used to identify these variants. The most relevant results are presented in Table 5, which comprises the studies in which novel AD-HL-related genes were found, studies where novel causative variants were detected in previously known genes, and descriptions of previously reported variants.

Novel candidate genes were revealed by the study of prominent Latin American families. For instance, the study of a family from Costa Rica allowed mapping and identifying *DIAPH1* as a novel non-syndromic progressive hearing loss gene (Lynch et al. 1997). *NCOA3* was recently indicated as a candidate gene to explain autosomal dominant hearing loss (Salazar-Silva et al. 2021). The association of *NCOA3* with HL was reinforced by the description of another likely pathogenic variant (NM_181659.3: c.2909G > C:p.(Gly-970Ala)) segregating with AD-NSHL in an Italian family (Tesolin et al. 2021). A genomic duplication mapped in chromosome 2 revealed the possible role of the overexpression *CNRIP1* and two lncRNA genes (*LOC107985892* and *LOC102724389*) in the etiology of hearing loss in a large Brazilian pedigree (Lezirovitz et al. 2020).

The relevance of CNVs to the etiology of non-syndromic hearing loss was investigated by Rosenberg et al. (2016). They used oligonucleotide array-CGH to investigate 50 cases of presumptive autosomal recessive inheritance and in 50 of presumptive autosomal dominant inheritance, in which *GJB2/GJB6* variants and m.1555A > G had been previously excluded. Rare copy number variants were detected in 12 subjects, but 4 were considered as probably causative (4%) because they comprised genes that have already been associated with HL and segregated with the phenotype. Two cases of the group with presumptive autosomal dominant inheritance were confirmed to have causative CNVs (2/50–4%) with dominant transmission.

Among these reports, the most striking was the variant c.2090 T > G: (p.Leu697Trp) in the *MYO3A* gene in Brazilian families. The majority of variants described in the *MYO3A* gene up to 2018 were associated with autosomal recessive hearing loss, making this variant a peculiar finding. Functional studies have permitted to explain the dominant transmission because of a dominant-negative effect (Dantas et al. 2018). Besides, the same variant was found in five apparently unrelated pedigrees, two in the original description of the variant (Dantas et al. 2018) and three in the recent report of Bueno et al. (2021). SNP array analysis followed by kinship analysis revealed that individuals from the five pedigrees were related and inherited a common haplotype of 607 kb. Furthermore, the variant had also been previously deposited in the LOVD Database by Dutch researchers, firstly as a VUS. The inclusion of the Dutch sample in the haplotype analysis revealed a shared chromosomal region of 87,121 bp between individuals from the six pedigrees, indicating the age of the most recent common ancestor and confirming a European origin of the mutation. The variant identification in Brazilian and Dutch patients allowed the speculation that the variant was introduced by Dutch colonists who occupied Northeastern Brazil in the seventeenth century and spread to the Southeastern region in the following centuries. The finding that the c.2090 T > G: p.(Leu697Trp) was present in a Dutch family, and about 1% of pedigrees with autosomal dominant hearing loss (Bueno et al. 2021) suggests that it may be frequent in other regions of Brazil and may even occur in other European countries if screened in larger samples.

Concluding remarks

Our review allowed us to draw some practical conclusions despite the severe limitations in the coverage of studies and the wide heterogeneity in investigation strategies. First, although *GJB2/GJB6* was confirmed to be a relevant cause of HL, the diagnostic rates of *GJB2/GJB6* studies are not the same in all geographical regions within Latin America. Second, the c.35delG, although frequent and significant as causing HL, was not always the most frequent causative variant in all samples, thus arguing against its screening being used as a single test.

We hypothesized that the probability of getting a molecular diagnosis with *GJB2/GJB6* testing in Latin America is inversely related with the proportion of Native American ancestry since we observed that in countries where Native American ancestry is higher (p. ex. Guatemala, and Nicaragua), diagnostic rate is less than 10%. Thus, the greater the European ancestry, the greater the likelihood that a molecular diagnosis will be achieved with *GJB2/GJB6* screening first.

Rare recurrent variants and heterogeneous patterns of variant distribution were described in this review. For instance, the c.2090 T > G:p.(Leu697Trp) *MYO3A* variant was shown to be a frequent cause of autosomal dominant postlingual progressive HL in Brazil. This type of information is helpful to guide molecular testing, mainly in centers with limited equipment and limited funding, where NGS is far from becoming a routine. Other frequent or recurrent causative variants would likely stand out if

comprehensive analyses using NGS or other advanced high throughput technologies could be applied to all of the diversely admixed countries of Latin America. In conclusion, Latin American studies contributed significantly to our current scientific knowledge of the genetic causes of HL, although with severe limitations to the study of many different genes. They showed the incredible value of studying highly admixed populations and point out a yet poorly explored potential in revealing new insights into hearing physiology. Besides, understanding the regional patterns of distribution of mutated alleles is of profound relevance to planning strategies for molecular studies in hearing loss, aiming to reduce costs and widen the range of patients and populations with access to efficient genetic counseling.

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Availability of data and material Additional data and material are available upon request.

Declarations

Conflicts of interest/Competing interests The corresponding author states that there is no conflict of interest on behalf of all authors.

Ethics approval The study was approved by the Institutional Ethics Committee (Biosciences Institute, University of São Paulo).

Consent to participate Written informed consent was obtained from participants or guardians of participants. The study was approved by the Institutional Ethics Committee (Biosciences Institute, University of São Paulo).

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