Facultad de Medicina

DEGREE IN MEDICINE

FINAL DEGREE PROJECT

Characteristics of hearing loss in patients with variants in the *COL2A1* gene (Stickler Syndrome type 1)

Características de la hipoacusia en pacientes con variantes del gen *COL2A1* (Síndrome de Stickler tipo 1)

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CONTENTS

ABBREVIATIONS

AC: air conduction

B: benign

BERA: Brainstem evoked response audiometry

BPPV: benign paroxysmal positional vertigo

CEIM: Comité de Ética para la Investigación con Medicamentos y Productos Sanitarios de Cantabria

CI: cochlear implant

CT: computerized tomography scan

dB: decibels

HL: hearing loss

HUMV: Hospital Universitario Marqués de Valdecilla

LB: likely benign

LP: likely pathogenic

MRI: magnetic resonance imaging

NGS: next-generation sequencing

OAE: otoacoustic emissions

P: pathogenic

SS: Stickler syndrome

STL1: Stickler syndrome type 1

VUS: variant of unknown significance

ABSTRACT

Introduction: Stickler syndrome type 1 (STL1) is an autosomal dominant genetic disorder, which affects the connective tissue. It is characterized by ocular, orofacial, skeletal, and auditory abnormalities. The aim of our study was to determine the variability of the auditory phenotype associated with pathogenic variants of the *COL2A1* gene and its impact on long-term treatment and follow-up.

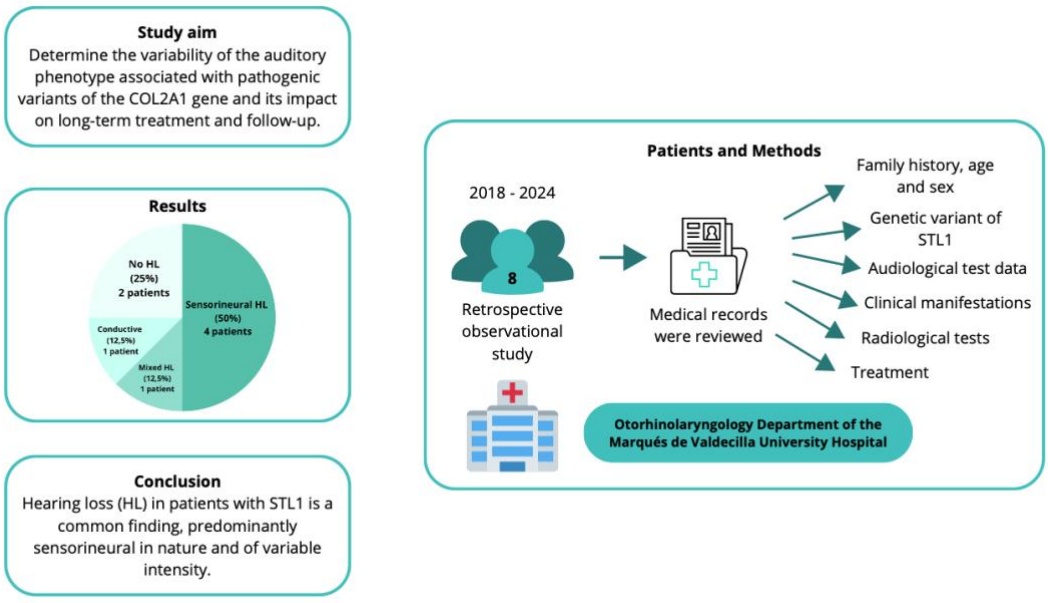
Patients and Methods: a retrospective observational study was conducted on patients with STL1 treated between 2018 and 2024 at the Otorhinolaryngology Department of the Marqués de Valdecilla University Hospital. The electronic medical records were reviewed to gather family history, age, sex, genetic variant of STL1, audiological test data, associated clinical manifestations, radiological tests, and treatment.

Results: Over the study period, 8 patients were identified with STL1, including 6 probands ad 2 familial cases. Six of 8 patients showed hearing loss (75%), ranging in severity from moderate-severe (n=1) to severe (n=3) and profound (n=2). In 4 cases (66.7%), were neurosensory, in 1 conductive (16.7%), and in another (16.7%) mixed. Only one patient required a cochlear implant with good progress regarding language comprehension.

Conclusion: Our study suggests that hearing loss in patients with STL1 is a common finding, predominantly sensorineural in nature and of variable intensity.

Keywords: *COL2A1*; Stickler Syndrome, autosomal dominant; genetic hearing loss; sensorineural hearing loss.

Graphical Abstract



RESUMEN

Introducción: el síndrome de Stickler tipo 1 (STL1) es un trastorno genético de herencia autosómica dominante que afecta al tejido conectivo. Se caracteriza por anomalías oculares, orofaciales, esqueléticas y auditivas. El objetivo de nuestro estudio fue determinar la variabilidad del fenotipo auditivo asociado a variantes patogénicas del gen *COL2A1* y su impacto en el tratamiento y seguimiento a largo plazo.

Pacientes y métodos: estudio observacional retrospectivo de los pacientes con STL1 atendidos entre 2018 y 2024 en el Servicio de Otorrinolaringología del Hospital Universitario Marqués de Valdecilla. Se revisaron las historias clínicas electrónicas para recoger los antecedentes familiares, edad, sexo, variante genética de STL1, resultados de pruebas audiológicas, manifestaciones clínicas asociadas, pruebas radiológicas y tratamiento.

Resultados: Durante el período de estudio se identificaron 8 pacientes con STL1, incluyendo 6 probandos y 2 casos familiares. Seis de los 8 pacientes (75%) mostraron pérdida auditiva, variando la gravedad de moderada-severa (n=1), severa (n=3), y profunda (n=2). En 4 (66.7%) la pérdida fue neurosensorial, en 1 de transmisión (16,7%), y en otro (16,7%) mixta. Solo un paciente precisó de un implante coclear con buena evolución en cuanto a la compresión del lenguaje.

Conclusiones: Nuestro estudio sugiere que la pérdida de audición en pacientes con STL1 es un hallazgo frecuente, predominantemente de tipo neurosensorial y de intensidad variable.

Palabras clave: *COL2A1*; Síndrome de Stickler; autosómico dominante; hipoacusia genética; hipoacusia neurosensorial.

INTRODUCTION

Stickler syndrome (SS), also known as hereditary progressive arthro-ophthalmopathy (ORPHA828), is a connective tissue disorder characterized by the presence of ocular, skeletal, orofacial, and auditory abnormalities. Typical features include vitreoretinal degeneration, high-grade myopia, retinal detachment, glaucoma, cataracts, premature osteoarthritis, midfacial hypoplasia, cleft palate, and hearing loss(1,2) (Figure 1). It is important to mention that SS is the most common cause of retinal detachment in childhood and familial retinal detachment, which can lead to blindness early in life (3).

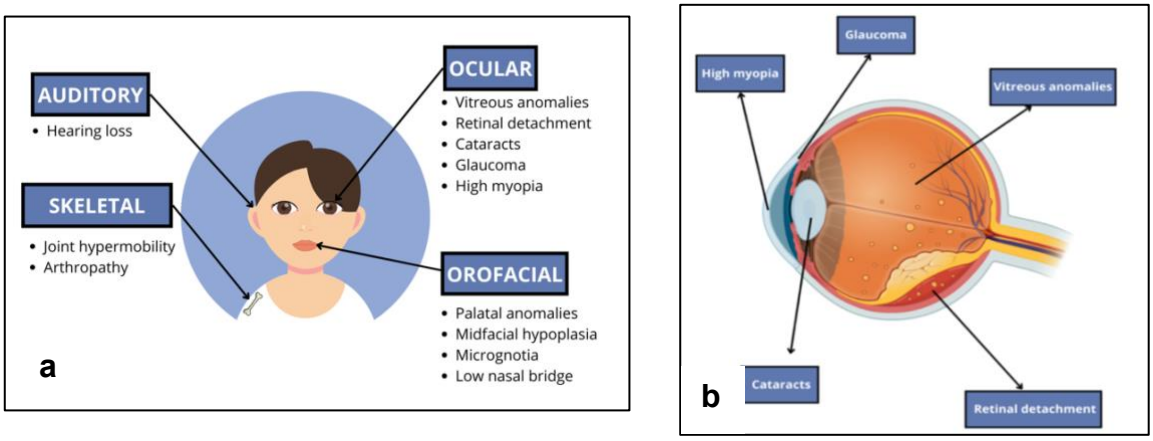


Figure 1. Clinical (a) and ocular (b) manifestations of Stickler Syndrome.

SS has a great phenotypic heterogeneity due to the wide variety of genes and mutations involved in the coding and assembly of type II, IX, and XI collagen (Figure 2). It can be inherited in both dominant (common) and recessive (rare) modes of inheritance (4–14).

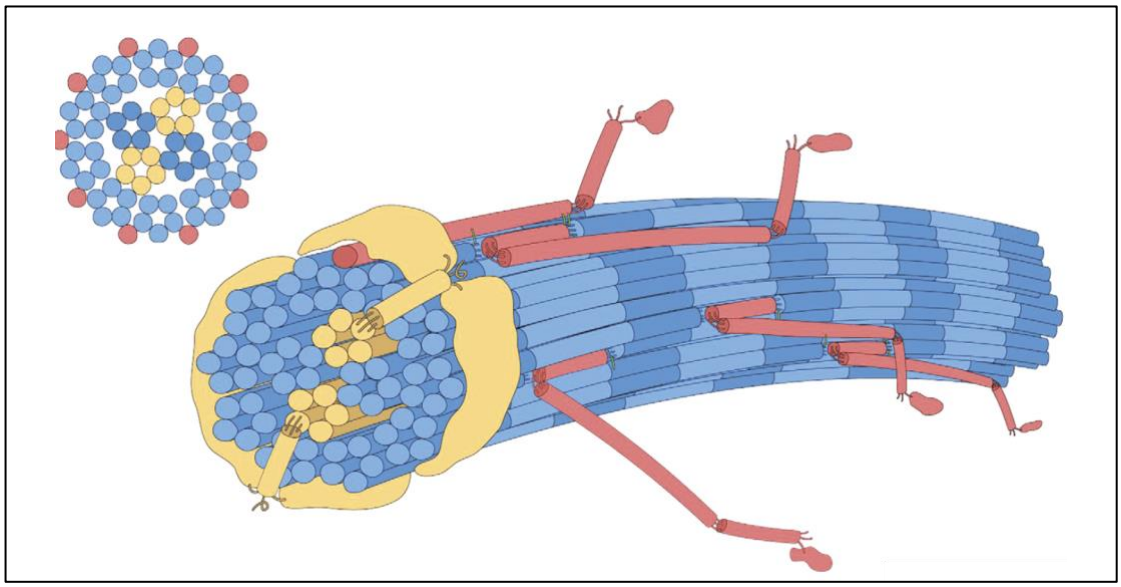


Figure 2. Schematic representation of the microfilament composition of collagen in cartilage. Blue: collagen II molecules; yellow: collagen XI molecules; red:

collagen IX molecules. Collagen IX molecules project outward and interact with the local environment. Adapted from Kadler, KE et al. (14)

The most common form is SS type 1 (STL1), which has an autosomal dominant inheritance pattern and is caused by heterozygous loss-of-function variants of the *COL2A1* gene, resulting in haploinsufficiency (3). In these patients, the most representative manifestations are ocular, specifically high-grade myopia and membranous vitreous, characterized by the persistence of vestigial vitreous gel in the retrolental space delineated by a membrane, and HL (1,2,7,15,16). As a result, STL1 patients are often diagnosed in Ophthalmology and Otorhinolaryngology consultations. Other manifestations of STL1 include palatal abnormalities (cleft palate, submucous cleft, high-arched palate) and joint pain (osteoarthritis), which is reported in 41% of patients before the age of 10 and in 90% of those over the age of 40(17).

The *COL2A1* gene encodes the α-1 chain of type II procollagen, which is composed of three identical chains folded into a helix. Mature type II collagen is created after the secretion of procollagen into the extracellular space and the cleavage of NH2 and COOH propeptides (18,19). This processed collagen will create a fibrillar network covalently interwined with the extracellular matrix to provide tension to the tissues where it is found (20,21). The mutations involved are primarily loss-of-function, with most predicted to result in mRNA degradation mediated by nonsense mutations (16). Glycine substitutions, which disrupt the triple helical structure of the collagen chain, can also cause STL1 and appear to be associated with an increased risk of HL (1) .

Type II collagen is the main component of hyaline articular cartilage and is also present in vitreous structures and the inner ear (22), specifically in inner and outer hair cells, as well as in the tectorial membrane (23). This localization of type II collagen explains why the most significant manifestations of STL1 are those previously mentioned. Additionally, due to the great interfamilial and intrafamilial phenotypic variability observed in STL1 patients, it is not possible to predict which patients will develop HL.

On the other hand, the auditory phenotype has been scarcely studied, and as such, the prevalence and characteristics of HL in STL1 patients are not well defined, potentially being conductive, sensorineural, or mixed (2).

The aim of our study was to determine the variability of the auditory phenotype based on *COL2A1* gene variants and their impact on long-term treatment and follow-up.

PATIENTS AND METHODS

Study design

This is a retrospective observational study of patients with variants in the *COL2A1* gene treated between January 2018 and December 2024 at the Otorhinolaryngology Department of the Marqués de Valdecilla University Hospital (HUMV) in Santander (Cantabria), a tertiary referral hospital of Cantabria that serves a population of approximately 570,000 inhabitants.

Variables

- For each individual, family and medical history, presence of dizziness/instability, tinnitus, and HL were assessed. HL data were collected from the clinical record, and it included onset age, mode of onset (abrupt or progressive), involvement (unilateral or bilateral, symmetrical or asymmetrical), progression (stable, fluctuating, or progressive) and other otologic symptoms (vertigo episodes, otalgia, or suppuration). Additionally, findings of systemic involvement were also noted.

- **Hearing**: The degree of HL in pure-tone audiometry was classified according to the criteria established by the American Speech-Language-Hearing Association (decibels, [dB HL]) (24):

    - **Normal hearing:** hearing thresholds from -10 to 15 dB.

    - **Slight hearing loss:** hearing thresholds from 16 to 25 dB. Difficulties may appear in hearing soft sounds or in noisy environments.

    - **Mild hearing loss:** hearing thresholds from 26 to 40 dB. Difficulty might occur in hearing soft sounds or following conversations in noisy environments.

    - **Moderate hearing loss:** hearing thresholds from 41 to 55 dB. At this level, it may be difficult to follow conversations at normal volume.

    - **Moderately-severe hearing loss:** hearing thresholds from 56 to 70 dB. Individuals with this level of hearing loss require amplification to comprehend conversations.

    - **Severe hearing loss:** hearing thresholds from 71 to 90 dB. Communication without specialized amplification is very limited at this level.

    - **Profound hearing loss:** hearing thresholds above 91 dB. People in this category cannot hear most sounds, even with conventional amplification, and may require specialized devices such as cochlear implants (CI).

In addition, configuration of HL as seen on audiometric analysis was classified as (25):

- **Descending**: HL is greater at high frequencies than at low frequencies. This type of hearing loss is common in presbycusis.
- **Flat**: HL is relatively uniform across all frequencies.
- **Ascending**: in this pattern, HL is greater at low frequencies than at high frequencies. It may be associated with some conditions such as Ménière's disease.
- **Mid-frequency:** HL is more pronounced at mid-frequencies, while low and high frequencies may be less affected. This type of hearing loss may indicate certain hereditary or congenital disorders.

Progression of hypoacusis was measured in the index cases using the mean difference in conversational thresholds in both ears in the first hearing test compared with the latest. An HL rate was calculated by dividing the mean loss of both ears between the years of follow up.

- It was also collected from the medical records, if they had performed brainstem evoked response audiometry (BERA), otoacoustic emissions (OAE), vestibular tests, computerized tomography scan (CT) of the temporal bones, and magnetic resonance imaging (MRI) of the cerebellopontine angles and/or posterior fossa.

- **Genetic analysis:** Sanger and Next Generation Sequencing (NGS) techniques were used. On one hand, the Sanger technique was used in patients with direct suspicion of SS, and on the other hand, the NGS technique was used in those with suspected genetic HL. In the case of the latter technique, after obtaining genomic DNA from peripheral blood, a massive sequencing study was carried out using a panel of genes associated with HL (Supplementary Material), which included the *COL2A1* gene. The sequencing workflow was performed by capturing genes of interest using RNA probes with SureSelect technology (Agilent Technologies, Inc. San Diego, California, USA) and subsequent sequencing with the Illumina Miseq sequencer (San Diego, California, USA).

- **Bioinformatic analysis** was performed using Alissa software, which integrates BWAmem programs for sequence alignment against the reference genome (hg19 assembly) and SAMtools to generate BAM files. Filtering of genetic variants was carried out according to alignment and genotyping quality metrics. Copy number variation detection was performed with an adapted version of the DECoN software (PMID:28459104). Variants identified from low-quality mapping alignments, chain-biased variants, as well as variants published as benign and likely benign by multiple submitters in ClinVar and HGMD databases were discarded. Variants were annotated using various databases containing functional (Ensembl, CCDS, RefSeq, Pfam), population (dbSNP, gnomAD, 1000 Genomes, ESP6500, ExAC),

disease-related (Clinvar, HGMD professional) information, as well as hereditary disease databases OMIM, Orphanet, and GeneReviews.

- **Variants were classified into 5 categories:** pathogenic (P), likely pathogenic (LP), variants of unknown significance (VUS), likely benign (LB), and benign (B), following ACMG recommendations, and expert specification of ACMG/AMP variant interpretation guidelines for genetic HL and variant interpretation. (PMID:30311386).

Data Collection

Patient data were obtained from the electronic medical record, utilizing both the old scanned record and information collected in the Altamira program. The documents were the clinical history and the reports of complementary tests. An Excel database was created with all the previously described variables. The patients´ identification data were dissociated and coded with an internal code that prevented direct patient identification. Only information relevant to the objective of the study was included.

Statistical Analysis

A descriptive statistical study of the cases included in the study was performed (IBM SPSS Statistics v22.0 statistical package for Mac).

Ethical Aspects

The study was conducted in compliance with Good Clinical Practice standards, in accordance with the principles of the World Medical Association Declaration of Helsinki and Spanish legislation. This study and its informed consent have been approved by the Research Ethics Committee for Medicinal Products and Health Products of Cantabria (CEIM, internal code 2024.425). The use of biological samples was carried out in accordance with current legislation on biomedical research (Law 14/2007, of July 3, on Biomedical Research).

RESULTS

During the 7-year study period, 8 patients were diagnosed with STL1 (6 probands and 2 family cases), of which 5 were women (62.5%) and 3 were men (37.5%), with ages at diagnosis ranging from 1 to 84 years. The demographic, clinical characteristics, allelic variants, and treatment are summarized in Table 1. In our study, 5 pathogenic or likely pathogenic variants and 1 variant of unknown significance (VUS) were found (F6/P1) (Table 1).

The reason for genetic study was due to suspicion of hereditary HL in 4 patients (50%), in one case (F1/P1) due to the craniofacial features presented, and in 3 cases (F1/P2, F1/P3, and F5/P1) as part of a family segregation study (F5/P1 had 3 children and 1 granddaughter diagnosed with SS in another Autonomous Community).

Hearing loss was observed in 6 of the 8 patients (75%), being sensorineural in 4 of them (66.7%), conductive in one (16.7%), and mixed in another (16.7%), and affecting both ears in all cases. In 3 patients, the HL was asymmetrical when comparing both ears (Figure 3, Table 1). Regarding severity, HL was moderately-severe in 1 patient (16.67%), severe in 3 patients (50%), and profound in 2 patients (33.34%). Additionally, 2 of the patients showed significant progression of HL compared to age-related physiological hearing deterioration. Regarding vestibular symptoms, only one patient with sensorineural HL (F2/P1) presented a condition compatible with benign paroxysmal positional vertigo (BPPV).

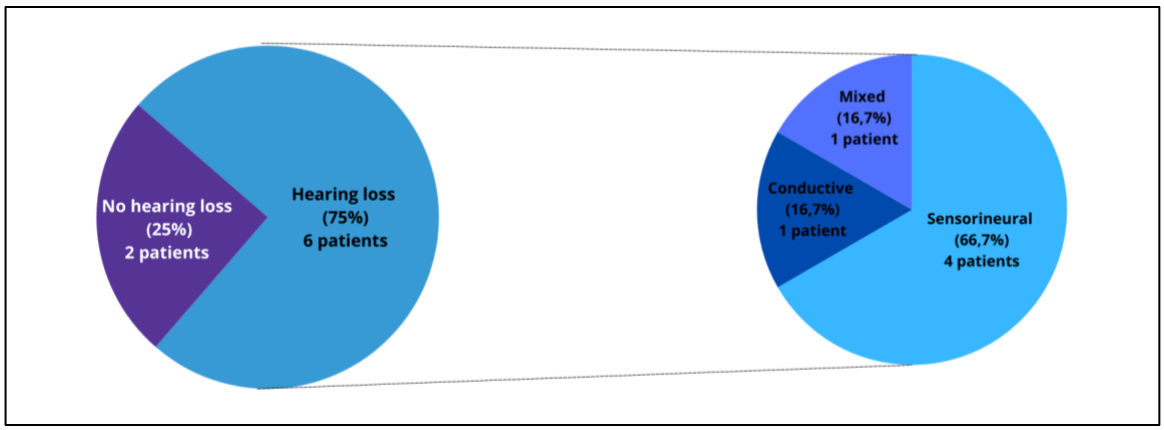


Figure 3. Prevalence and type of hearing impairment in our series.

Furthermore, 6 of the 8 patients presented other typical manifestations of STL1 due to collagen involvement at other levels. This associated pathology was varied among the different patients and included manifestations at the ocular, articular, and palatal levels (Table 1). A palatal defect was present in 37.5% (3/8) of the studied population. Among the 3 patients with a palatal defect, 2 exhibited HL: one presented conductive HL, while the other demonstrated sensorineural HL. (Table 1).

Regarding treatment, the 4 patients with sensorineural HL required hearing aids, and only one of them a cochlear implant (CI), which improved auditory symptoms and language comprehension (F3/P1) (Table 1). On the other hand, it is noteworthy that the only patient with conductive HL (F1/P2) had cholesteatomatous otitis media, which improved auditory symptoms after surgical intervention (tympanoplasty + mastoidectomy and contralateral transtympanic drainage). Therefore, in our series, all these therapeutic interventions had a favorable outcome, improving the auditory symptoms of patients followed in the consultation of the Otorhinolaryngology Department at HUMV.

Table 1. Demographic, clinical, genetic characteristics, and treatment of patients with STL1.

Patient	Sex	Age	Reason for study	Family history	Age of Onset	Progression	Type of Hearing Loss and Auditory Profile	Degree of Hearing Loss	Vestibular Symptoms	Associated Pathology	CT/MRI	Genetic Variant / Protein	Treatment
F1/P1	Male	1 year	Craniofacial features	Yes	NA	NA	Transient serous otitis. Normal hearing afterward. Passes OAE and BERA at birth	No loss	No	Myopia Cleft palate	Not performed	c.1468_1475delinsT	Not required
F1/P2	Male	50 years	Family Segregation Study	Yes	0-10 years	No	Flat Conductive Hearing Loss	RE: Moderate LE: Severe	No	Cleft palate	Mastoid Occupation. Cholesteatomatous COM	c.1468_1475delinsT	Middle Ear Surgery
F1/P3	Female	84 years	Family Segregation Study	Yes	NA	NA	No hearing loss	No loss	No	Vitreous Anomalies Myopia	Not performed	c.1468_1475delinsT	Not required
F2/P1	Female	60 years	Hearing Loss Under Investigation	No	50 years	Uncertain	Descending Sensorineural Hearing Loss	RE: Severe LE: Moderate-Severe	Benign Paroxysmal Positional Vertigo	Bilateral RD Osteoarticular	Not performed	c.816+1G>A	Bilateral Hearing Aids
F3/P1	Male	50 years	Hearing Loss Under Investigation	Yes	20-30 years	Yes	Descending Sensorineural Hearing Loss	RE and LE: profound	No	Cleft palate Osteoarticular Cataratcs	Normal CT and MRI	c.3583G>T (p.Glu119S)	Hearing Aids Unilateral CI
F4/P1	Female	56 years	Hearing Loss Under Investigation	Yes	44 years	Yes	Descending Sensorineural Hearing Loss	RE: mild LE: profound	No vertigo V-HIT: normal VNG: unilteral hypofunction	No	Normal CT	c.1833+1G>A COCH too	Hearing Aids
F5/P1	Female	58 years	Family Segregation Study	Yes	40-50 years	NA	Bilateral Symmetrical Sensorineural Hearing Loss with Flattened Curves	RE y LE: moderate-severe	No	Glaucoma Osteoarticular	Not performed	c.1783delG, p.Ala595Leufs*34	Hearing Aids
F6/P1	Female	31 years	Hearing Loss Under Investigation	Yes	6 years	NA	Mixed Hearing Loss	RE: mild LE: severe	No	No	CT and MRI: Dilation of the Left Endolymphatic Sac	c.3111+5G>A (VUS)	Not required

***Abbreviations**: F: Family / P: Proband / OAE: Otoacoustic Emissions / BERA: Brainstem evoked response audiometry / RE: Right Ear / LE: Left Ear / V-HIT: Video Head Impulse Test / VNG: Videonystagmography / RD: Retinal Detachment / CT: Computerized Tomography scan / MRI: Magnetic Resonance Imaging / COM: Chronic Otitis Media / VUS: Variant of Unknown Significance / CI: Cochlear Implant / NA: Not Available.*

DISCUSSION

During the nearly 7-year study period, 8 patients were retrospectively identified as carriers of mutations in the *COL2A1* gene, with 3 of them belonging to the same family (Figure 4, Table 1).

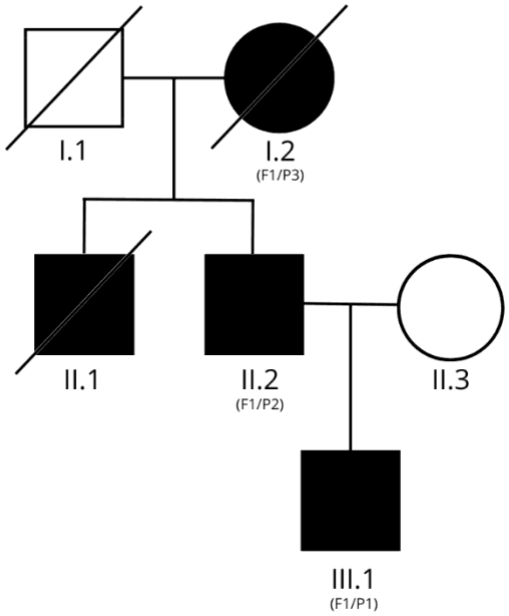


Figure 4. Family tree of the family 1.

Table 2 shows a systematic review of the literature on auditory manifestations in patients with STL1. The incidence of HL in different articles ranges between 17.2% and 74% (2,13,16,26,27). This variation could be due to the different ages of the patients included in the reviews, being lower in those that include younger patients and higher as age increases, since possible HL produced by STL1 adds to physiological degenerative HL associated with aging. In our study, 6 of the 8 patients had HL (75%), of which 4 were sensorineural, 1 was conductive, and 1 was mixed (Table 1, Figure 3). Both the high incidence and severity of HL in our study may be due to the fact that this was the reason for the study of 4 of our probands. Both in our study and in the reviews (2,16), it seems that the predominant HL is of the sensorineural type.

Table 2. Auditory manifestations in patients with STL1. Systematic review of the literature.

	Author	Year	Country	Systematic Review or Case Series	Number of Cases Included	Hearing Loss (%)	Type of Hearing Loss (%):
1	Acke F et al.	2012	Belgium	Systematic Review	224	52,20%	SN: 66,2% C: 13,4% M: 19,92%
2	Hoornaert KP et al.	2010	Belgium	Case Series	100	74%	SN: 68,9% C: 31,1%
3	Terhal PA et al.	2015	Netherlands	Case Series	93	17,20%	
4	Bath F et al.	2021	United States	Case Series (pediatrics)	16	25%	SN: 25%. C: 25% M: 25% NA:25%
5	Choi S et al.	2021	Korea	Case Series	30	30%	

***Abbreviations**: SN: Sensorineural / C: Conductive / M: Mixed / NA: Not Applicable.*

Sensorineural HL associated with STL1 has been characterized by some authors as bilateral, mild, and for high frequencies (1,2,28), with a non-significant progression compared to physiological age-related hearing impairment (28). In our study, 4 patients with P/LP variants had sensorineural HL, of which 1 was moderately-severe, 1 was severe, and 2 were profound (F2/P1, F3/P1, F4/P1, and F5/P1) (Table 1), and their audiometric tests are shown in Figure 5. These data seem to indicate that there is more heterogeneity regarding severity than previously reported.

The proband F2/P1 is a 60-year-old woman who was studied for severe sensorineural HL without a family history. A study with NGS was carried out and the analysis revealed the c.816+1G>A mutation, which is described in ClinVar (NM_001844.5(*COL2A1*): c.1023+1G>A) (29) as pathogenic. This variant was expressed in F2/P1 in addition to HL, with bilateral retinal detachment, osteoarticular manifestations, and BPPV (Table 1).

Meanwhile, F3/P1 is a 50-year-old male for whom a genetic study was decided due to profound bilateral sensorineural HL. In addition to this clinical picture, the patient had a cleft palate, osteoarticular manifestations, and cataracts. All these manifestations are compatible with STL1, and moreover, the LP mutation c.3583G>T in the *COL2A1* gene was demonstrated. This patient also showed a significant progression of HL that cannot be attributed to presbycusis.

The proband F4/P1 is a 56-year-old woman who was studied for profound sensorineural HL without other associated manifestations and normal imaging tests. When the genetic analysis was performed, mutations were observed in both the *COCH* gene (OMIM: 603196) and the *COL2A1* gene (c.1833+1G>A). This variant is defined as P in ClinVar (NM_001844.5(*COL2A1*):c.1833+1G>A) (30), despite this, F4/P1 did not present other manifestations associated with STL1. Because this patient had mutations in both the *COCH* (also associated with progressive sensorineural HL), and the *COLA2A1* gene, we cannot determine which gene was responsible for the auditory phenotype that was found.

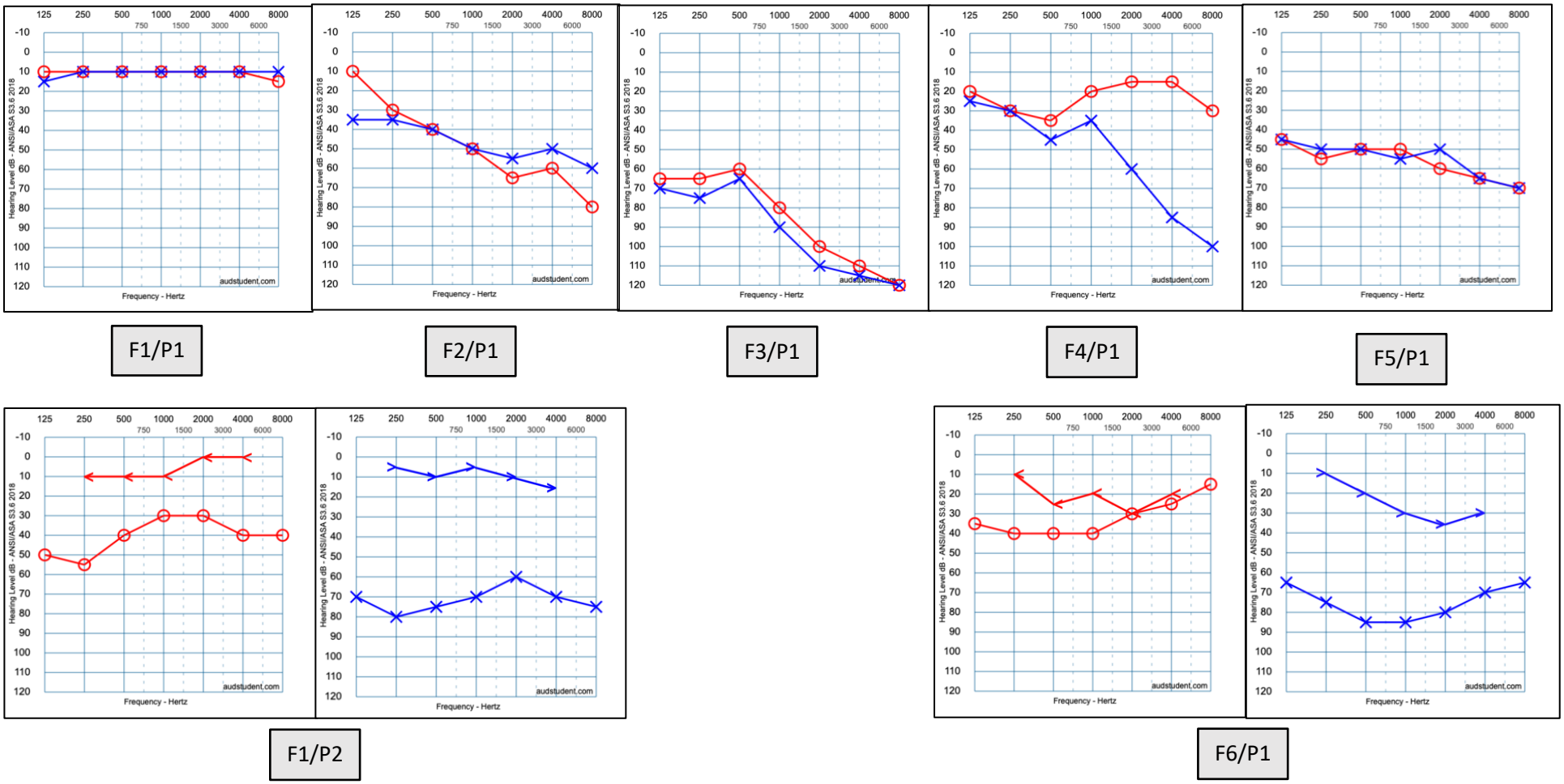


Figure 5. Audiometric test of the patients included in the study. Red: right ear. Blue: left ear. In F1/P1, the air conduction (AC) and normal hearing are represented. In F2/P1, F3/P1, F4/P1, and F5/P5, the AC is represented, reflecting sensorineural HL. In F1/P2 and F6/P1, both AC and bone conduction (BC) are represented, with the former case being conductive HL and the latter being mixed

In the last patient with sensorineural HL in our study (F5/P1), as in the previous probands, the genetic analysis was conducted to study the HL, revealing the c.1783delG mutation. In addition to these symptoms, the patient had osteoarticular manifestations and glaucoma, leading us to conclude that the mutation is LP.

The 4 previously mentioned patients in our series with sensorineural HL required adaptation of hearing aids. In the case of F3/P1, a cochlear implant (CI) was added due to lack of response to the hearing aid, with good results in language discrimination.

Typically, the associated HL is not detected in neonatal screening programs, as middle frequencies tend to remain intact in cases of inner ear involvement, so the onset timing in these cases remains unclear, but it is believed to be in childhood(1). Moreover, the amplitude of distortion product otoacoustic emissions, a test of outer hair cell function, has been found to be significantly decreased, more than expected according to the pure-tone audiogram (28).

Continuing with conductive and mixed HL, this is much less common in patients with STL1, most of whom are pediatric patients, and is predominantly due to middle ear problems, especially if there is a history of cleft palate and associated Eustachian tube dysfunction, where these problems can persist into adulthood(1,2,13). In our series, there is a proband (F1/P2) with severe bilateral conductive HL, in addition to palatal defects. The study of this patient began from F1/P1, a one-year-old patient with a cleft palate and myopia. Suspecting a possible genetic defect, the Sanger technique was used, discovering a P variant in the *COL2A1* gene, located on chromosome 12. These findings are compatible with the diagnosis of STL1, and therefore the study of both F1/P2, father of F1/P1, and F1/P3, grandmother of F1/P1, was decided upon, finding that all three shared the c.1468_1475delinsT mutation and the inheritance pattern was autosomal dominant (Figure 4). The three probands exhibited anomalies compatible with STL1 at different levels; F1/P1 had the previously mentioned myopia and cleft palate, F1/P2 also had a cleft palate, and F1/P3 had myopia and vitreous anomalies. At the auditory level, F1/P1 presented severe serous otitis media with normal hearing after the resolution of the episode; and F1/P2 had severe bilateral conductive HL with a flattened audiometry (Figure 5), and radiological images compatible with chronic cholesteatomatous otitis media, which was treated with mastoidectomy for cholesteatoma removal and contralateral transtympanic drainage.

From this information, it can be deduced that the c.1468_1475delinsT mutation has pathological significance, since 100% of the patients carrying it in our study present signs of STL1, although each of them presented a different phenotype. Close monitoring of F1/P1 is important to prevent ocular and auditory manifestations that may develop over the years.

The last patient in our series is F6/P1, a 36-year-old woman with mild mixed HL in the right ear and severe HL in the left ear (Figure 5), who also presented a dilation of the left endolymphatic sac demonstrated on CT and MRI. The mutation found (c.3111+5G>A) is defined as VUS in ClinVar (NM_001844.5(*COL2A1*):c.3111+5G>A)(31). Because of this, and the absence

of any other pathology associated with the STL1, we cannot conclude whether the HL is genetic or secondary to the anatomic anomaly demonstrated radiologically.

Although the occurrence of otosclerosis in patients with STL1 has been reported, it is more likely that is a random association rather than a characteristic of STL1, since otosclerosis is highly prevalent in the general population(1,15,28).

Hypermobility of the tympanic membrane has also been described in SS, including type 1(28,32,33). This can be associated with visibly hyperflaccid tympanic membranes by otoscopy or with normal tympanic membranes. In the first group, it was not possible to differentiate whether the cause was recurrent or chronic episodes of otitis media and the use of transtympanic drainage tubes, or the dysfunction of type II collagen in STL1. In those with normal membranes, hypermobility of the ossicular joints was proposed as the cause, as hypermobility in other joints is also observed in patients with SS (32).

Although one of our patients (F2/P1) reported BPPV, we do not have data to assert vestibular involvement in STL1. This has not been previously reported in the medical literature, although considering BPPV episodes can appear in other patients with genetic HL (34), we cannot rule it out in our case either.

Finally, it is important to note that although our work focuses on auditory pathology, STL1 manifests at other levels, mainly ocular and articular (1,2,5,15,16). Therefore, it is essential to take this into account in order to stablish both an early diagnosis of suspicion, as well as eye, hearing and skeletal system monitoring protocols that allow early treatment to be stablished.

The main limitation of our study is that it is a descriptive work with very few diagnosed cases of STL1. However, given the scarcity of previous studies addressing HL in these patients and the disparity of their results, our series provides valuable insights that complement what has previously been published.

CONCLUSIONS

HL associated with STL1 is a common finding and highly variable, both in type, with sensorineural being the most frequent, and in intensity, ranging in our series from moderately-severe to profound cases that may require cochlear implants (CI). In our study, the response to the CI in the only case that needed it was favorable in terms of language comprehension.

Our study indicates the importance of a personalized diagnosis and therapeutic approach, given the phenotypic variability of STL1, to enhance clinical management and long-term follow-up of these patients. It also highlights the need for comprehensive audiological evaluation in all patients diagnose with STL1 to facilitate early identification of HL and to tailor therapeutic interventions accordingly.

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SUPPLEMENTARY MATERIAL

Genes included in the panel NGS. The methodology of the external laboratory is described in Cabanillas et al.(35)

External Laboratory (2018-2019)

Genes (n): 229

ABHD12, ACTB, ACTG1, ADGRV1, AIFM1, ALMS1, AMMECR1, ANKH, AP1S1, ATP1A3, ATP6V0A4, ATP6V1B1, BCAP31, BCS1L, BRAF, BSND, CABP2, CACNA1D, CCDC50, CDH23, EACAM16, CHD7, CIB2, CISD2, CLCNKA, CLCNKB, CLDN14, CLPP, CLRN1, COCH, COL2A1, COL4A3, COL4A4, COL4A5, COL4A6, COL9A1, COL9A3, COL11A1, COL11A2, COLEC11, DCAF17, DDX11, DIABLO, DIAPH1, DNMT1, ECHS1, EDN3, EDNRB, EPS8L2, ESPN, ESRRB, EYA1, EYA4, FGF3, FGFR3, FTO, GATA3, GIPC3, GJB2, GJB3, GJB6, GPSM2, GRHL2, GRXCR1, GSDME, HARS1, HARS2, HGF, HOMER2, HOXA1, HOXB1, HSD17B4, ILDR1, KARS1, KCNE1, KCNJ10, KCNQ1, KCNQ4, LARS2, LHFPL5, LHX3, LOXHD1, LRP2, LRTOMT, MARVELD2, MASP1, MIR96, MITF, MSRB3, MT-CO1, MT-RNR1, MT-TH, MT-TK, MT-TL1, MT-TS1, MYH9, MYH14, MYO3A, MYO6, MYO7A, MYO15A, NARS2, NDP, NLRP3, OPA1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX3, PCDH15, PDZD7, PEX1, PEX2, PEX3, PEX5, PEX6, PEX26, PJVK, POGZ, POU3F4, POU4F3, PRPS1, PTPN11, PTPRQ, RAF1, RDX, RMND1, SALL1, SERAC1, SERPINB6, SIX1, SLC17A8, SLC19A2, SLC26A4, SLC33A1, SLC52A2, SLC52A3, SLITRK6, SMPX, SNAI2, SOX10, SPATA5, STRC, SYNE4, TBC1D24, TECTA, TIMM8A, TJP2, TMC1, TMEM132E, TMIE, TMPRSS3, TPRN, TRIOBP, TRPV4, TSPEAR, USH1C, USH1G, USH2A, WFS1, WHRN, XYLT2, ADCY1, AP3D1, ATP2B2, ATP6V1B2, BDP1, CCS, CD151, CD164, CDC14A, CLIC5, COL9A2, COQ6, CRYM, DCDC2, DIAPH3, DSPP, ELMOD3, EPS8, ERAL1, EXOSC2, FBLN1, FGFR1, FGFR2, FOXI1, GRXCR2, GSTP1, GTF2IRD1, HMX2, HMX3, KITLG, MAF, MAFB, MARS2, MCM2, MT-CO3, MT-TE, MT-TS2, NDUFA13, NFIX, PANX1, PMP22, PNPT1, POLD1, PSIP1, PTPRD, RAI1, RIPOR2, ROR1, S1PR2, SEMA3E, SIX5, SLC4A11, SLC9A1, SLC22A4, SLC26A5, SLC44A4, TBL1XR1, TK2, TMPRSS5, TNC, TUBB4B, TWIST1, WBP2, YWHAH

HUMV´s 1st panel (2020-2021)

Genes (n): 188

ABHD12, ACTB, ACTG1, ADCY1, ADGRV1, AIFM1, ALMS1, ANKH, AP1S1, ATP1A3, ATP2B2, ATP6V1B1, ATP6V1B2, BCAP31, BCS1L, BDP1, BRAF, BSND, CABP2, CACNA1D, CCDC50, CDH23, CEACAM16, CHD7, CIB2, CISD2, CLCNKA, CLCNKB, CLDN14, CLPP, CLRN1, COCH, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, COL4A6, COL9A1, COL9A2, COL9A3, COQ6, CRYM, DCAF17, DCDC2, DDX11, GSDME, PJVK, DIABLO, DIAPH1, DIAPH3, DNMT1, DSPP, ECHS1, EDN3, EDNRB, ELMOD3, EPS8, EPS8L2, ESPN, ESRRB, EYA1, EYA4, RIPOR2, FBLN1, FGF3, FGFR1, FGFR2, FGFR3, FOXI1, FTO, GATA3, GIPC3, GJB2, GJB3, GJB6, GPSM2, GRHL2, GRXCR1, GRXCR2, GTF2IRD1, HARS2, HGF, HMX2, HMX3, HOMER2, HOXA1, HOXB1, HSD17B4, ILDR1, KARS, KCNE1, KCNJ10, KCNQ1, KCNQ4, KITLG, LARS2, LHFPL5, LHX3, LOXHD1, LRP2, LRTOMT, MAF, MARS2, MARVELD2, MASP1, MCM2, MIR96, MITF, MSRB3, MYH14, MYH9, MYO15A, MYO3A, MYO6, MYO7A, NARS2, NDP, NDUFA13, NFIX, NLRP3, OPA1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX3, PCDH15, PDZD7, PEX1, PEX2, PEX26, PEX3, PEX5, PEX6, PNPT1, POU3F4, POU4F3, PRPS1, PTPN11, PTPRQ, RAF1, RDX, RMND1, SEMA3E, SERAC1, SERPINB6, SIX1, SIX5, SLC17A8, SLC19A2, SLC26A4, SLC26A5, SLC33A1, SLC4A11, SLC52A2, SLC52A3, SLC9A1, SLITRK6, SMPX, SNAI2, SOX10, SPATA5, STRC, SYNE4, TBC1D24, TECTA, TIMM8A, TJP2, TK2, TMC1, TMEM132E, TMIE, TMPRSS3, TMPRSS5, TNC, TP63, TPRN, TRIOBP, TSPEAR, USH1C, USH1G, USH2A, WFS1, WHRN y XYLT2.

HUMV´s 2nd panel (2022-2023)

Genes (n): 231

ABHD12, ACTB, ACTG1, ADCY1, ADGRV1, AIFM1, ALMS1, AMMECR1, ANKH, AP1S1, ATP1A3, ATP2B2, ATP6V0A4, ATP6V1B1, ATP6V1B2, BCAP31, BCS1L, BDP1, BRAF, BSND, BTD, CABP2, CACNA1D, CCDC50, CD164, CDC14A, CDH23, CEACAM16, CEP78, CHD7, CHSY1, CIB2, CISD2, CLCNKA, CLCNKB, CLDN14, CLDN9, CLICK5, CLPP, CLRN1, CAR, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, COL4A6, COL9A1, COL9A2, COL9A3, COQ6, CRYM, DCAF17, DCDC2, DDX11, DEVIL DIAPH1, DIAPH3, DLX5, DMXL2, DNMT1, DSPP, ECHS1, EDN3, EDNRB, ELMOD3, EPS8, EPS8L2, ERAL1, ESPN, ESRRB, EYA1, EYA4, FBLN1, FDXR, FGF3, FGFR1, FGFR2, FGFR3, FITM2, FOXI1, FTO, GAB1, GATA3, GIPC3, GJB2, GJB3, GJB6, GPRASP2, GPSM2, GRAP, GREB1L, GRHL2, GRXCR1, GRXCR2, GSDME, GTF2IRD1, HARS2, HGF, HMX2, HMX3, HOMER2, HOXA1, HOXA2, HOXB1, HSD17B4, ILDR1, KARS, KCNE1, KCNJ10, KCNQ1, KCNQ4, KITLG, KMT2D, LARS2, LHFPL5, LHX3, LMX1A, LOXHD1, LRP2, LRTOMT, MAF, MAN2B1, MANBA, MARS2, MARVELD2, MASP1, MCM2, MGP, MIR96, MITF, MPZL2, MSRB3, MYH14, MYH9, MYO15A, MYO3A, MYO6, MYO7A, NARS2, NDP, NDUFA13, NFIX, NLRP3, OPA1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX1, PAX3, PCDH15, PDE1C, PDZD7, PEX1, PEX2, PEX26, PEX3, PEX5, PEX6, PJVK, PLS1, PNPT1, POU3F4, POU4F3, PPIP5K2, PRPS1, PTPN11, PTPRQ, RAF1, RAI1, RDX, REST, RIPOR2, RMND1, ROR1, S1PR2, SEMA3E, SERAC1, SERPINB6, SIX1, SIX5, SLC17A8, SLC19A2, SLC26A4, SLC26A5, SLC33A1, SLC44A4, SLC4A11, SLC52A2, SLC52A3, SLC9A1, SLITRK6, SMPX, SNAI2, SOX10, SPATA5, SPNS2, STRC, SUCLA2, SYNE4, TBC1D24, TBL1X, TECTA, TIMM8A, TJP2, TK2, TMC1, TMEM126A, TMEM132E, TMIE, TMPRSS3, TMPRSS5, TNC, TP63, TPRN, TRIOBP, TRRAP, TSPEAR, TUBB4B, TWNK, USH1C, USH1G, USH2A, WBP2, WFS1, WHRN y XYLT2.