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Clinical characterization and molecular genetics of vestibular migraine patients

Caracterización clínica y genética molecular de pacientes con migraña vestibular

INTERNATIONAL PhD THESIS

TESIS DOCTORAL CON MENCIÓN INTERNACIONAL

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GRANADA

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ABBREVIATURES

ACMG American College of Medical and Genomics

AF   Allele Frequency

AICA   Anterior Inferior Cerebellar Artery

AMP   Association for Molecular Pathology

ASMES   Asociación Síndrome de Meniere España

BAM   Binary Alignment Map

BBB   Blood Brain Barrier

BPPV Benign Paroxysmal Positional Vertigo

BWA-MEM   Burrows-Wheeler Aligner's Maximal Exact Matches algorithm

CADD Combined Annotation Dependet Depletion

CAM   cell adhesion molecules

CGAS Candidate Gene Association Studies

CGRP Calcitonin Gene-Related Pepride

CMT   Charcot-Marie-Tooth demyelinating

CNS   Central Nervous System

CNV   Copy Number Variants

CSVS Collaborative Spanish Variant Service

CT   Computed Tomography

dbHL   decibels Hearing Level

DFNA deafness, autosomal dominant

EAC   External Auditory Canal

EAC   Episodic ataxia

EMT   epithelial-mesenchymal transition

ENT   Ear Nose Throat

ESIT-SQ   European School for Interdisciplinary Inventory Screening Questionnaire

FHM   Familial Hemiplegic Migraine

FPKM Fragments Per Kilobase of exon per Million

GABA gamma-aminobutyric acid

GBA   Gene Burden Analysis

gEAR Gene Expression Analysis Resource

gnomAD   Genome Aggregation Database

GTEx   Genotype-Tissue Expression

GÜF   Geräuschüberempfindlichkeit test

GWAS Genome-Wide Association

HADS Hospital Anxiety and Depression Scale

HADS-A   Hospital Anxiety and Depression Scale-anxiety

HADS-D   Hospital Anxiety and Depression Scale- depression

HCs   Hair Cells

ICHD   International Classification of Headache Disorders

IHC   Inner Hair Cells

IHS   International Headache Society

InDels Insertions and Deletions

LoF   Lost of Function

LOFTEE   Loss-Of-Function Transcript Effect Estimator

LVS   Large Structural Variants

MAF   Minor Allele Frequency

MAPK mitogen-activated protein kinase

MD   Meniere's Disease

ML   Machine Learning

MML   Minimum Masking Level

MRI   Magnetic Resonance Imaging

NFE   Non-Finnish European

OHC   Outer Hair Cells

OR   Odds Ratio

pLI   probability of being Loss of function-intolerant).

PTA   Pure Tone Average

RI   Residual Inhibition

RNA-seq   RNA sequencing

RPKM Reads Per Kilobase of transcript per Million

SAM   Sequence Alignment Map

SC   Semicircular Canals

SCA   Spinocerebellar Ataxia

SCAR Spinocerebellar Ataxia, autosomal recessive

SD   Standard deviation

SGN   spiral ganglion neurons

SHIELD   Shared Harvard Inner-Ear Laboratory Database

SNHL Sensorineual Hearing Loss

SNPs Single Nucleotide Polymorphims

SNV   Single Nucleotide Variants

SVA   Single-Variant Analyses

SVs   Structural Variants

TFI   Tinnitus Functional Index

THI   Tinnitus Handicap Inventory

TPM   Transcripts Per Million

TRI   Tinnitus Research Initiative

TVS   Trigeminovascular System

VEMP Vestibular-evoked Myogenic Potential

VEP   Variant Ensamble Predictor

VGN   vestibular ganglion neurons

v-HIT Video Head Impulse Test

VM   Vestibular Migraine

VMo   Vestibular Migraine without tinnitus

VMt   Vestibular Migraine with tinnitus

VOR   Vestibular-Ocular Reflex

VQSLOD   Variant Quality Score Log-odds

WES   Whole Exome Sequencing

WHO   World Health Organization

WS   Waardenburg Syndorme

ABSTRACT

Tinnitus, the perception of sound without an external source, is a multifaceted condition with significant variability in its demographic and clinical presentations. Vestibular migraine (VM) is characterized by concurrent activation of vestibular and cranial nociceptive pathways. Despite its prevalence, the mechanisms of VM remain poorly understood, and a potential genetic contribution has yet to be fully described. This thesis aimed to identify patients with VM and tinnitus, examine the relationship between hyperacusis and tinnitus in VM and explore genetics through exome sequencing studies.

First, a cross-sectional study assessed the profile of 434 chronic tinnitus patients using the ESIT-SQ, comparing online and hospital responses via age-based clustering. Next, we estimated tinnitus and hyperacusis prevalence in 51 VM patients, analyzing their association with hearing loss, anxiety, and depression through audiological, psychoacoustic, and psychometric assessments. Finally, we analyzed exome sequencing datasets in four families with multiple VM cases.

In the tinnitus cohort, we found significant differences in education level, tinnitus-related hearing disorders, sleep difficulties, dyslipidemia, and various tinnitus characteristics, partially adjusted for age. In the VM cohort, 75% of patients reported tinnitus, most commonly at 8000Hz, with no difference in hearing thresholds between those with and without tinnitus. Hyperacusis was present in 60% of VM patients and was linked to higher THI scores and increased anxiety and depression. Familial VM showed significant symptoms variability, even within the same family. Whole exome sequencing identified numerous variants shared among affected family members, including two missense mutations in family 4 in the ANK3 gene.

Accordingly, this thesis emphasizes the limited reliability of self-reported tinnitus surveys for phenotyping and the need for further clinical evaluations. The age-based clustering of clinical profiles provides a valuable tool for comparing ESIT-SQ responses among tinnitus subgroups. The prevalence of tinnitus among VM patients, independent of standard audiogram hearing loss, and its significant association with hyperacusis, anxiety, and depression could have profound implications for patient care. Finally, exome sequencing through familial studies may identify new candidate genes and offer further insights into VM pathophysiology.

RESUMEN

Los acúfenos son la percepción de sonido sin una fuente externa, presentan una variabilidad significativa en sus manifestaciones. La migraña vestibular (MV) se caracteriza por la activación simultánea de vías vestibulares y nociceptivas craneales. A pesar de su prevalencia, los mecanismos de la MV y su posible contribución genética no se comprenden completamente. Esta tesis tiene como objetivo identificar pacientes con MV y acúfenos, explorar la relación entre hiperacusia y acúfenos en la MV, y examinar la genética a través de estudios de secuenciación del exoma.

Primero, se realizó un estudio transversal para analizar el perfil de 434 pacientes con acúfenos crónicos usando el ESIT-SQ, comparando respuestas en línea y en el hospital según la edad. Luego, se estimó la prevalencia de acúfenos e hiperacusia en 51 pacientes con MV, analizando su relación con pérdida auditiva, ansiedad y depresión mediante evaluaciones audiológicas y psicométricas. Finalmente, se analizaron datos de secuenciación del exoma en cuatro familias con casos múltiples de MV.

En la cohorte de acúfenos, se encontraron diferencias significativas en el nivel educativo, trastornos auditivos relacionados, dificultades para dormir y dislipidemia, ajustadas por edad. En la cohorte de MV, el 75% de los pacientes reportaron acúfenos, comúnmente a 8000Hz, sin diferencias en umbrales auditivos. La hiperacusia se presentó en el 60% de los pacientes con MV, asociándose con mayor ansiedad y depresión. La MV familiar mostró variabilidad significativa de síntomas. La secuenciación del exoma identificó variantes compartidas en el gen ANK3 en una familia.

Esta tesis resalta la limitada fiabilidad de las encuestas autoinformadas de acúfenos y la necesidad de evaluaciones clínicas adicionales. La agrupación por edad proporciona una herramienta valiosa para comparar subgrupos de acúfenos. La alta prevalencia de acúfenos entre los pacientes con MV y su asociación con hiperacusia, ansiedad y depresión tiene importantes implicaciones clínicas. Finalmente, la secuenciación del exoma puede identificar nuevos genes y ofrecer más información sobre la fisiopatología de la MV.

1. INTRODUCTION

The ear is the sensory organ responsible for hearing sounds and balance in humans. Its anatomy is complex; it comprises three parts: outer or external ear, middle ear, and inner ear. To understand the pathophysiological bases that underline Vestibular migraine (VM), it is essential to explain the anatomy and function of the ear (Figure 1).
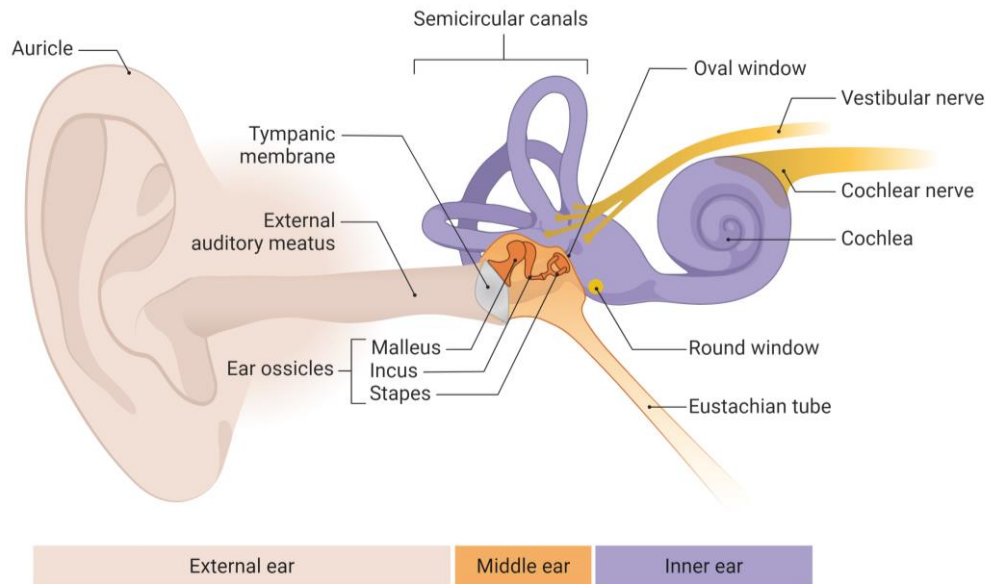
Figure 1. Ear Anatomy. *Created with BioRender.com*

- The outer ear is composed of the auricle and the ear canal. The auricle, which consists of a cartilage structure covered by skin, collects sound waves and allows them to converge in the external auditory canal (EAC). This canal conducts the sound waves towards the tympanic membrane and, as a resonant effect, allows frequencies between 2000 and 50000Hz to be reinforced [1,2].

- The middle ear, an air-filled space between the tympanic membrane and the oval window of the inner ear, contains three small-interconnected bones: the malleus, the incus, and the stapes. These ossicles transmit and amplify the sound waves from the tympanic membrane to the inner ear [1,3].

- The inner ear is in the temporal bone. It is where the most complex processes of signal perception and transformation occur, both balance and auditory mechanisms.

1.1. Inner Ear Anatomy

The inner ear is located within the petrous portion of the temporal bone and is composed of a bony and a membranous labyrinth. Two different fluids flow through the inner ear: the perilymph, which is between the bony and membranous labyrinths, and the endolymphatic fluid, which is located within the membranous labyrinth[4]. The inner ear

has two main components: the cochlea and the vestibule, which are responsible for hearing function and the sense of equilibrium, respectively [3] (Figure 2).

The inner ear's connections with the extraocular, cervical, trunk, and limb muscles are necessary for maintaining balance [1,5].
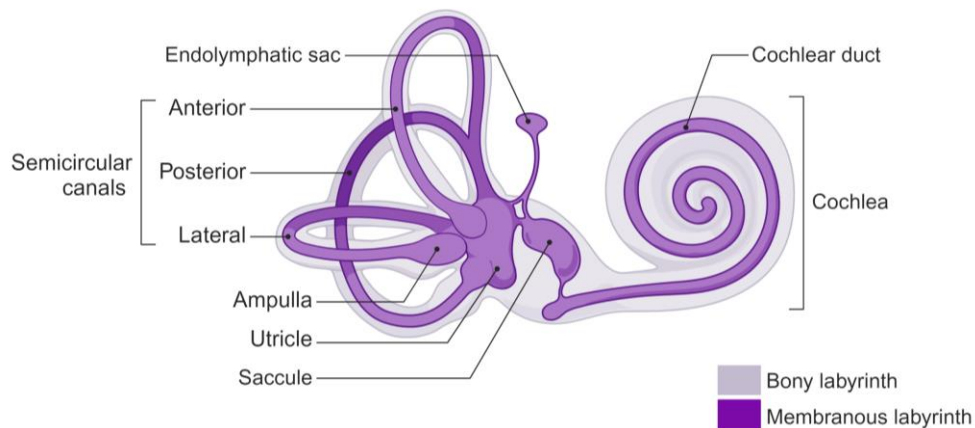
Figure 2. Inner ear anatomy. *Created with BioRender.com*

1.1.1. Bone Labyrinth

The bony structure protects and contains the membranous labyrinth. The structures of the bony labyrinth are filled with perilymph that is continuous with and similar in composition to the cerebral spinal fluid. The perilymphatic duct into the adjacent subarachnoid space drains this fluid[6]. The bony labyrinth is divided into two parts:

1.1.1.1. Cochlea or anterior labyrinth

It corresponds to the anterior part of the labyrinth[7]. The cochlea is a spiral-shaped structure, which consists of a cubic conic tube with a longitude of 30 mm and a diameter of 1-2 mm, and it consists of two and a half turns with an anterolateral direction around a central axis, named modiolus. The base of the modiolus is located close to the internal acoustic meatus, where the branch of the cochlear portion of the vestibulocochlear nerve enters[1]. The spiral lamina, a thin osseous lamina, extends along the modiolus. This lamina continues with the basilar membrane, keeping the cochlear duct centered[8].

1.1.1.2. Vestibular system or posterior labyrinth

It is responsible for maintaining equilibrium based on the detected angular and linear accelerations of the head and gravitational forces acting upon the body[4].

The vestibular apparatus comprises the *vestibule*, where the utricle and saccule are situated, and *semicircular canals* (SC) [7].

- The <u>Vestibule</u> is the central part of the bony labyrinth, communicating with the cochlea in its anterior part and with the SCs posteriorly [1] Three orifices are drilled into the vestibule, the oval window, the ampulla, and the posterior hole of the lateral SC.

- The <u>Utricle</u> and <u>Saccule</u> are located within the vestibule. These structures detect linear and gravitational acceleration because of the sensory neuroepithelium called the macula. The macula is oriented vertically in the saccule, and in the utricle, it is oriented horizontally [9]. The vestibular aqueduct runs from the vestibule through the temporal bone to the posterior part of the petrous portion of the temporal bone [1].

- Three bony <u>SCs</u> exist on each inner ear, with one horizontal and two vertical canals. These canals are in the posterior and superior parts of the labyrinth. The vertical canals are perpendicular to the lateral canal [8]. These canals are oriented nearly orthogonally to one another, allowing them to detect angular motion in any plane and direction. The SC planes on each side of the head are complementary so that head movements are detected and signaled by a pair of functionally coplanar canals [9]. At the end of each canal exists a dilation, the ampulla. It contains the vestibular sensory epithelium [8].

1.1.2. <u>Membranous Labyrinth</u>

The membranous labyrinth within the bony labyrinth contains the sensory epithelium. This structure transduces sound and head motion in the cochlea and vestibule, respectively [9]. The membranous labyrinth is organized into five structures: one cochlear duct, three semicircular ducts, and the otolithic organ, formed by the utricle and saccule[1].

1.1.2.1. Cochlea

The cochlea is a spiral-shaped auditory sensory organ, and the cochlear duct is enclosed in the bony structure of the cochlea [9]. The cochlear duct generates two distinct ducts, namely *Scala vestibuli* and *Scala tympani*, which traverse the cochlear spiral and interconnect at the apex, forming a specialized structure known as the *helicotrema* [1] (Figure 3).

These structures are disposed in a triangular shape. The *Scala vestibuli,* on the upper side, is separated from the *Cochlear duct (or Scala media)* by the Reissner's membrane. In contrast, the *Scala tympani*, on the lower side, and the *Cochlear duct* are separated by the basilar membrane, where we find the organ of Corti[10].

The *Scala tympani* and the *Scala vestibuli* are filled with perilymph. At the same time, the *Scala media* contains the endolymph secreted by the *stria vascularis*, a network of capillaries in the spiral ligament[9]. This fluid is similar in composition to intracellular fluid[6].
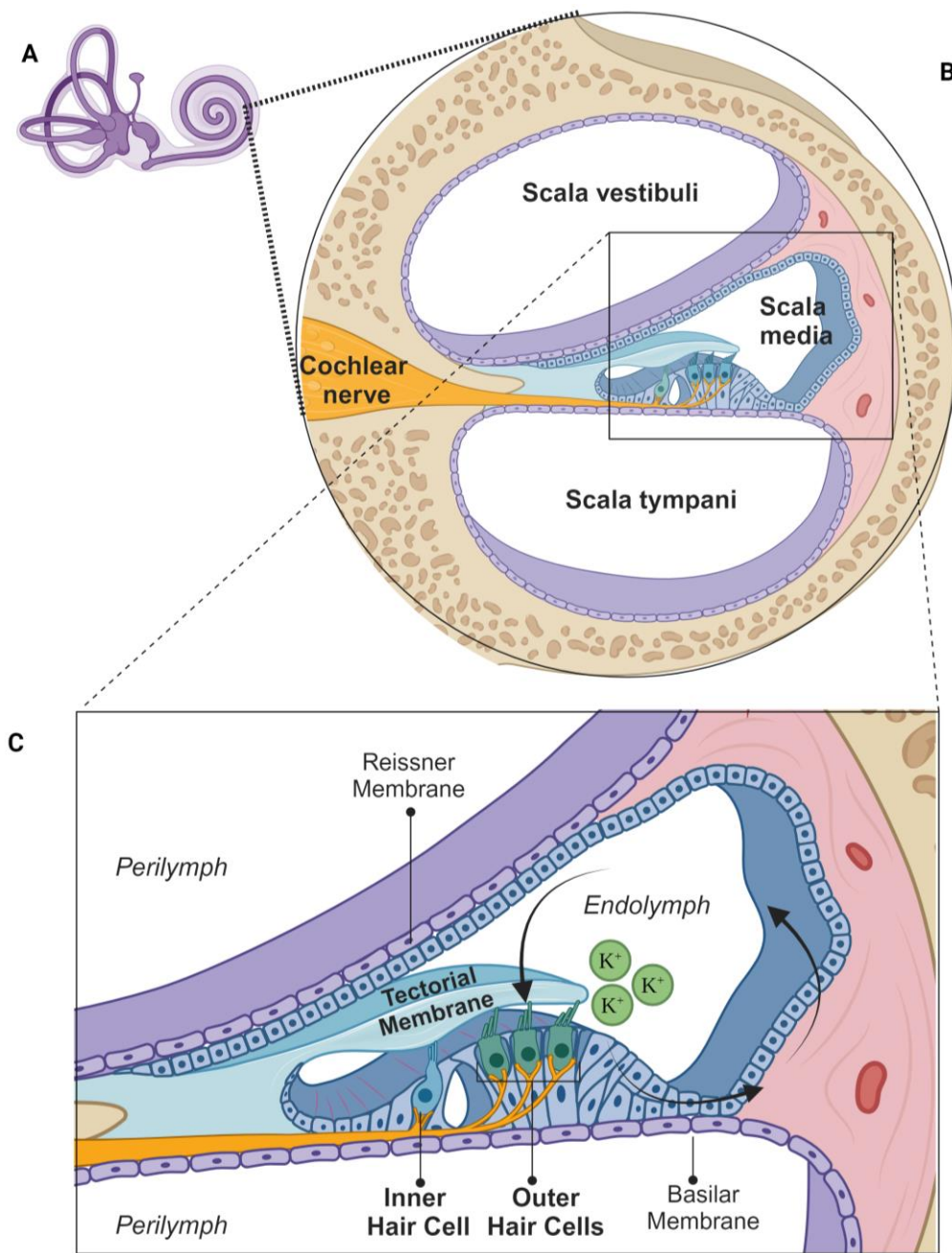
Figure 3. **Inner ear** (A), a **cross-section of the cochlea (**B) and **organ of Corti** (C).
In the organ of Corti, the K$^+$ from the endolymph is transported into the hair cells, depolarizing them. Then, the K+ is transported through the supporting cells to the stria vascularis, where the canals return it to the endolymph.
Created with BioRender.com.

- Organ of Corti

The organ of Corti, situated in the cochlear duct, is the sensory organ that transforms the mechanical vibration of the basilar membrane into neural signals. The organ of Corti rests over the basilar membrane, which can vibrate in response to fluid movements and propagate this mechanical energy to the entire organ of Corti[11]. The basilar membrane exhibits tonotopic organization and varies in stiffness along its length. The base of the membrane, closest to the stapes, is more rigid and tighter, responding preferentially to high-frequency sounds. In contrast, the apex of the basilar membrane is broader and more flexible, responding better to low-frequency sounds[12].

The organ of Corti contains three rows of outer hair cells (OHC) and one row of inner hair cells (IHC) that have a tonotopic arrangement through the cochlea, which allows to distinguish sounds and varying frequencies [13]. These hair cells (HCs) have stereocilia and kinocilia contacting with the tectorial membrane, which consists of a fibrous surface layer and a deep gelatinous layer located upper of the organ of Corti. The movement of these structures triggers the depolarization of the HCs and the release of neurotransmitters that act at the spiral ganglion[10].

- Hair Cells

Hair cells present several common characteristics, but they also have functional differences. The hair bundle, a mechanically sensitive organelle consisting of dozens of stereocilia, is located on the apical surface of HCs. In the OHCs, this hair bundle is attached to the tectorial membrane, while the hair cell bodies form tight connections with supporting cells that adhere to the basilar membrane at their basal surface[14].

As a result of the shear motion, the stereocilia of both types of HCs undergo bending, thereby inducing an influx of ionic current into the IHCs and OHCs, consequently modulating their membrane potentials. Regarding the IHCs, the stimulation triggered by deflected stereocilia leads to the generation of action potential transmitted to the auditory afferent nerve through synaptic connections. This process ultimately facilitates sound perception and recognition[13].

On the other hand, OHCs contract and elongate in response to their changes in membrane potential. This functional behavior of the OHCs is believed to increase the displacement amplitude of the organ of Corti, which is named *cochlear amplification*. This mechanism plays a crucial role in the heightened sensitivity, extensive dynamic range, and precise frequency selectivity exhibited by the auditory system[13].

Therefore, the information about the acoustic environment is primarily conveyed through synapses at the IHCs, whereas OHCs are involved in sound amplification through electromechanical feedback[11].

1.1.2.2. The otolithic system: utricle and saccule

There are two sets of otolith organs per side: the utricle and the saccule. They respond to linear acceleration, gravitational forces, and head-tilting [4]. Head movements stimulate a neuroepithelium called the macula. Thanks to the macula, the utricle detects movements in the horizontal plane. At the same time, the saccule's macula senses motion in the vertical plane[6].

The macula is covered by a gelatinous membrane containing small calcium carbonate particles known as otoliths or otoconia. These particles project vestibular receptor hair cells through the membrane.[6] The otoliths sense head linear acceleration[4]. As the otoliths are denser than endolymph, gravity deflects the stereocilia of HCs of the utricle and saccule when the head is stationary. The macula is adaptable, so when the stimulus of head tilting persists, the HCs bend and the depolarized membrane potentials gradually revert to their normal state. This restoration enables the HCs to respond to other positional changes[6].

1.1.2.3. Semicircular ducts

The semicircular ducts have the same basic structure as the bony SCs in which they are contained. The planes of the SCs on each side of the head are complementary, resulting in a pair of functionally coplanar canals that jointly provide complementary signals for head movements[4].

The semicircular ducts open into the utricle. At the end of each duct is a dilation called the ampulla, containing the *crista ampullaris*, histologically similar to the macula. This structure is within the cupula, a gelatinous substance in which HCs are embedded; however, it is thicker than the macula and does not contain otoliths[6]. What makes the cupula bend is the pressure exerted by the endolymph during head rotation. The flow of endolymph, which leads to excitation in one semicircular duct, will concurrently inhibit the hair cells of the contralateral duct with which it is paired[6].

1.1.2.4. Vestibular Hair Cells

As stated above, the vestibular system has two sensory neuroepithelium types: the macula and *crista ampullaris*. Both systems contain HCs composed of a large kinocilium

and 70-100 stereocilia apically. These are arranged so that the tallest stereocilia are closer to the kinocilium and the shortest are furthest away[6].

Human vestibular HCs can be classified according to their afferent synaptic terminal. Type I HCs are innervated by flask-shaped calyces and Type II HCs are innervated by boutons. Type I HCs are associated with irregular afferents with variable resting discharge. Meanwhile, Type II HCs tend to synapse on regular afferents with low variability resting discharge and are more common. Both present efferent connections from the vestibular nuclei[6].

1.1.3. Vascularization of the Inner Ear

1.1.3.1. Arterial system

The bone labyrinth is vascularized through the *inferior tympanic artery*, *stylomastoid artery,* and the *anterior vestibular artery*, which irrigates part of the utricle, the saccule, and the anterior and lateral SCs [1] (Figure 4).

On the other hand, the irrigation of the membranous labyrinth depends on the *internal auditory artery*, which is usually a branch of the anterior inferior cerebellar artery (AICA) or the basilar artery. The internal auditory artery gives rise to a branch known as the *anterior vestibular artery*, which supplies the anterior and horizontal SCs and the utricle. The internal auditory artery continues as the common cochlear artery bifurcated into two branches. The first is the *vestibulocochlear artery*, which supplies blood to the inferior SC, the saccule, and a part of the cochlea. The other branch is the *main cochlear artery*, which provides blood to most cochlea [4].
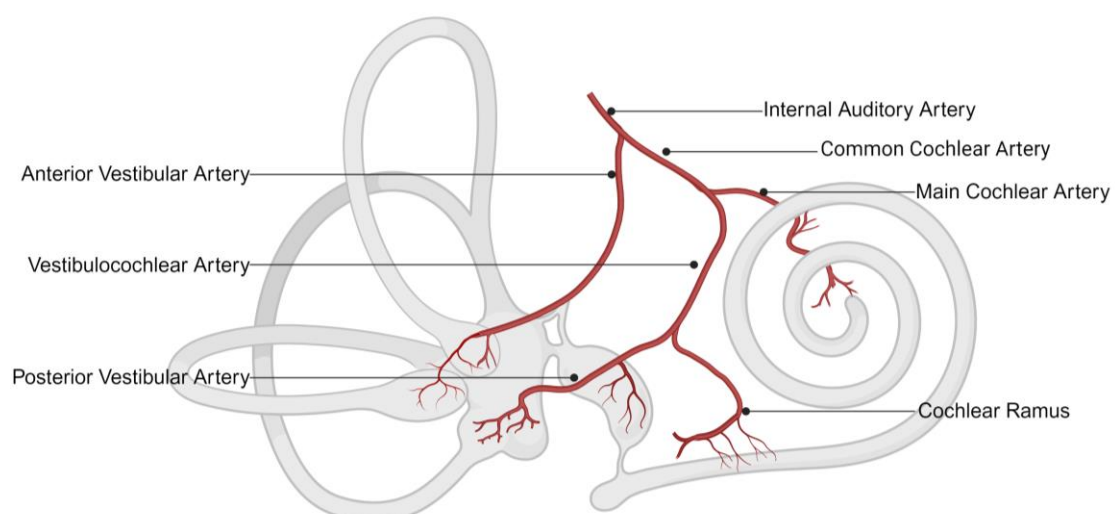
Figure 4. Arterial irrigation of the inner ear. *Created with BioRender.com*

1.1.3.2. Venous drainage

The vestibular and cochlear veins drain the membranous labyrinth, which runs parallel to their respective arteries. These veins converge to form the labyrinth vein, emptying into the sigmoid sinus[1].

The vestibular aqueduct's venous system is formed by the vestibular aqueduct vein, which runs along the aqueduct to the inferior petrosal sinus. The venous system of the cochlear aqueduct includes all the veins that drain to the cochlear aqueduct vein. From there, drainage continues to the inferior petrosal sinus and the jugular gulf[7].

1.1.3.3. Innervation

Each SC and otolith organ receives afferent innervation (singular nerves) from the vestibular nerve. These singular nerves are organized into inferior and superior components in which neuronal bodies are in the Scarpa ganglion.

The superior vestibular nerve contains fibers from the superior and horizontal canals and the utricle, while the inferior vestibular nerve contains fibers from the inferior canals and the saccule. The vestibular nerve consists of axons originating from the neurons of the Scarpa ganglion. These axons enter the pores of the internal acoustic meatus, positioned posteriorly to the cochlear nerve, forming the vestibulocochlear nerve or the VIII cranial nerve [4] (Figure 5)
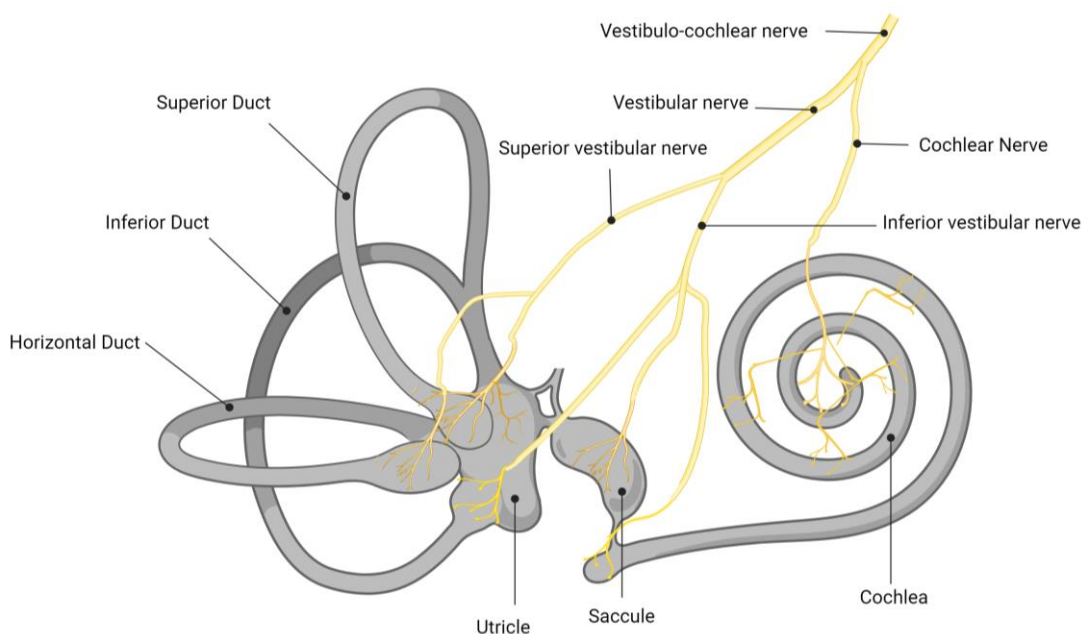
Figure 5. Innervation of the inner ear. *Created with BioRender.com*

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1.2. Inner Ear Function

The inner ear is an organ with two different functions: the auditory function, performed by the cochlea, and the vestibular function, by the vestibule.

1.2.1. Auditory Function

Each component of the human ear serves a specific function in sound perception. When sound waves reach the head, the auricle captures and directs them through the external auditory canal (EAC) toward the tympanic membrane, aiding in sound localization. The EAM also enhances the resonance of sound waves. Upon reaching the tympanic membrane, the vibrations are transmitted through the auditory ossicles to the oval window as mechanical energy. The organ of Corti in the cochlea is the specialized organ responsible for converting these acoustic signals (mechanical energy) into electrical impulses that are subsequently transmitted to the central nervous system (CNS) for interpretation[15].

The hearing organ allows the perception of physical sound stimuli in three stages:

- The physical energy of the sound stimulus captured in the pinna is transmitted or conducted to the organ of Corti.
- The mechanical energy is then transformed into electrical energy within the organ of Corti through *transduction*.
- Finally, the electrical energy is sent through nerve pathways from the organ of Corti to the auditory areas of the temporal lobe in the cerebral cortex[2].

1.2.2. Vestibular Function

Maintaining equilibrium relies on the continuous interplay between the vestibular, proprioceptive, and visual mechanisms, which are integrated and modulated in several CNS levels to achieve a normal balance. Dysfunction in these mechanisms can cause balance issues or hinder the recovery of otherwise uncomplicated vestibular disorders[4].

Vision, proprioception, and vestibular inputs are essential to human balance. When the connection between these systems is lost, vestibular disorders result in unpleasant symptoms of vertigo, oscillopsia, imbalance, and ataxia. Vestibulo-ocular, vestibulocollic and vestibulospinal connections are indispensable for maintaining the balance [5].

As explained above, the vestibule-dependent balance aspect is accomplished through intricate interactions between the otolith organs and SCs.

1.2.2.1. Semicircular Canals function

Ewald's Laws established the relationship between the planes of SCs, the direction of the endolymphatic flow, and the direction of the induced movements of the head and eyes at the end of the 19th century[16].

> 1) The direction of nystagmus is perpendicular to the stimulated SCs.
> 2) The velocity of nystagmus is proportional to the velocity of head movement.
> 3) The amplitude of nystagmus is proportional to the duration of head movement.

Thanks to the orthogonal positioning of the SCs, it is possible to detect any angular movements in any plane and direction. The semicircular canal planes are complementarily on each side of the head so that a pair of functionally coplanar canals complementarily signal head movements[4].

- The horizontal or lateral canals sense horizontal head rotation.
- Diagonal or oblique head movements are detected through the combined activation of an anterior canal on one side and a posterior canal on the other.

The horizontal SC is in the horizontal plane; the anterior SC is situated in the frontal plane, almost perpendicular to the petrous part of the temporal bone. Finally, posterior SC is placed in the sagittal plane[17].

SCs have an open end, which communicates freely with the vestibule, and a closed (cupular) end. When a head movement bends the sensory hair cells in the cupula, it generates bioelectrical activity and potentials down the vestibular nerve. As a result of activating the numerous separate canals, the CNS can determine the rotation plane. The frequency rate of the action potentials in the vestibular nerve, which in turn depends on the severity of the endolymph-induced cupular defection, provides information on the rotation's speed [4].

1.2.2.2. Function of the otolith organs
The otoliths detect linear acceleration in the head. The otoliths can detect a head tilt in relation to the gravity vector since gravity is a linear acceleration. The utricle and the saccule are the two pairs of otolith organs found on each side, which are displaced perpendicularly to each other[4].

The thick otolith membrane is 'left behind' when the head speeds up, deflecting HCs and causing action potentials in the vestibular afferents. The utricles are sensitive to linear accelerations in the horizontal plane because of their roughly horizontal orientation. Since the sacculi are roughly parasagittal oriented, they are susceptible to linear accelerations in the sagittal (vertical) plane [4].

The mechanism of activation of vestibular system is shown in the figure 6.
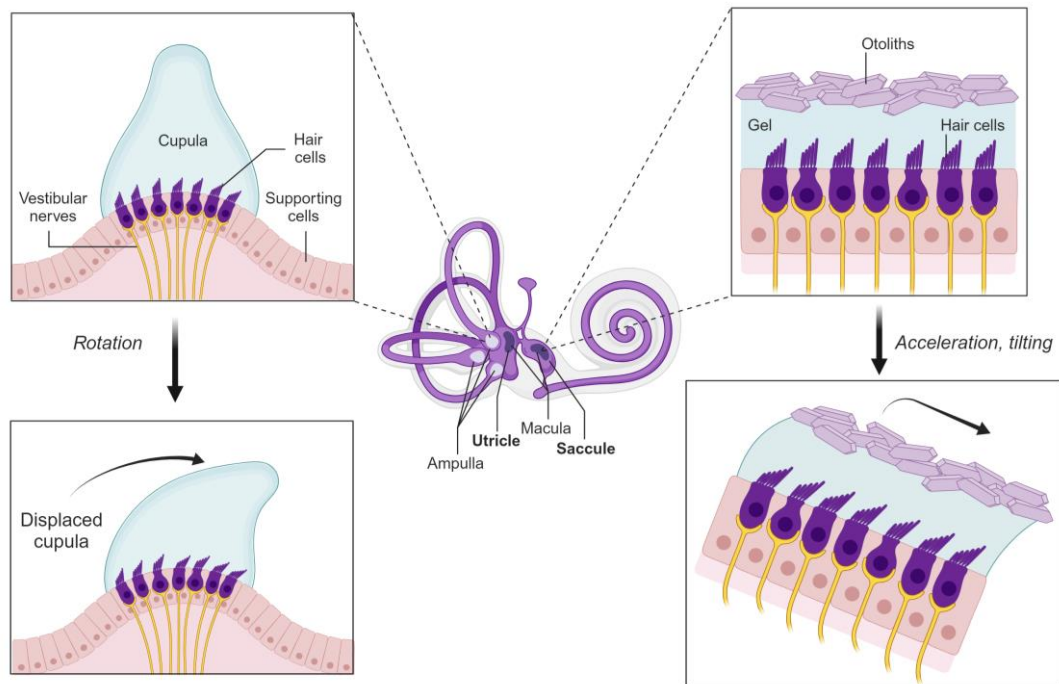
Figure 6. Morphological and vestibular sensory epithelia cellular structure, including hair cells, and the movement that produces the depolarization of the hair cells. *Created with BioRender.com*

1.3. Genetics

Genetics is the study of heredity and the variation of inherited qualities, specifically how particular traits are handed down from parents to kids based on the sequence of their DNA[18]. The genome is an individual's whole DNA sequence, which is organized into functional units, the genes. There are around 18500 genes in the human genome, and each gene consists of coding (exons) and non-coding regions (introns)[19].

The term "exome" comprises all the coding regions included in all the genes, which represent less than 2% of the genome[20].

1.3.1 Genetic Variants

A reference human genome sequence is an established, high-quality, and widely accepted human genome sequence used to compare DNA sequences generated in research. A variant is a permanent alteration in the DNA sequence that differs from that in the reference genome. Sequencing technologies allow us to identify the genetic variants of an individual or cohort with a particular pathology. These variations can be

inherited or appear spontaneously, and they play a crucial role in shaping individuals' features and susceptibility to illnesses.

There are several types of genetic variants, including single nucleotide variants (SNV), short Insertions and Deletions (InDels), copy number variants (CNV), and large structural variants (LSV).

1.3.1.1   *The single nucleotide variants*

Modifying a single base pair is called a SNV, the most common variant type, as shown in figure 7A.

SNVs are classified as codifiers or non-codifiers based on whether they affect codifying protein regions. Coding SNV can be non-synonymous if there is an amino acid change or synonymous if there is a nucleotide change but no amino acid change due to genetic code degeneration. Non-synonymous SNVs, also known as Lost of function (LoF) variants, occur when an amino acid change causes the appearance or disappearance of a stop/start codon or missense if the change produces an amino acid substitution[21,22].

1.3.1.2   *Short Insertions and Deletions*

InDels represent genetic variants characterized by the insertion or deletion of nucleotides within the DNA sequence, figure 7 B. These variants can be classified in frameshift if the mutation in a gene refers to the insertion or deletion of nucleotide bases in numbers that are not multiples of three[23], as the entire gene sequence following the mutation would be read incorrectly. If the indel is composed of a multiple of three, it does not change the reading frame; these variants are called in-frame insertion, deletion, or non-frameshift[22,24,25].

1.3.1.3   *Structural variants*

Structural variants (SVs) are events where a DNA region shows changes in the number of copies, orientation, or location. These genomic variations could affect larger chromosomal regions, and they involve at least 50 nucleotides to many thousands of nucleotides as inversions and translocations. If a structural variant modifies the total nucleotide count, it is categorized as a CNV. These encompass various alterations such as insertions, deletions, duplications, or tandem repeats, as shows figure 7 C. Moreover, beyond variants affecting protein-coding regions, alterations exist within non-coding regions like promoters, splice sites, and other regulatory elements[24].

The variant's allele frequency (AF) may be determined by comparing it to a reference population. Variants with an AF less than 0.01 are considered rare variants, and they are called single nucleotide polymorphisms (SNPs). Variants with an AF of 0.01 to 0.05 are regarded as less common, whereas variations with an AF greater than 0.05 are considered common. In addition, variations that have not been characterized in the population are called novel [22].
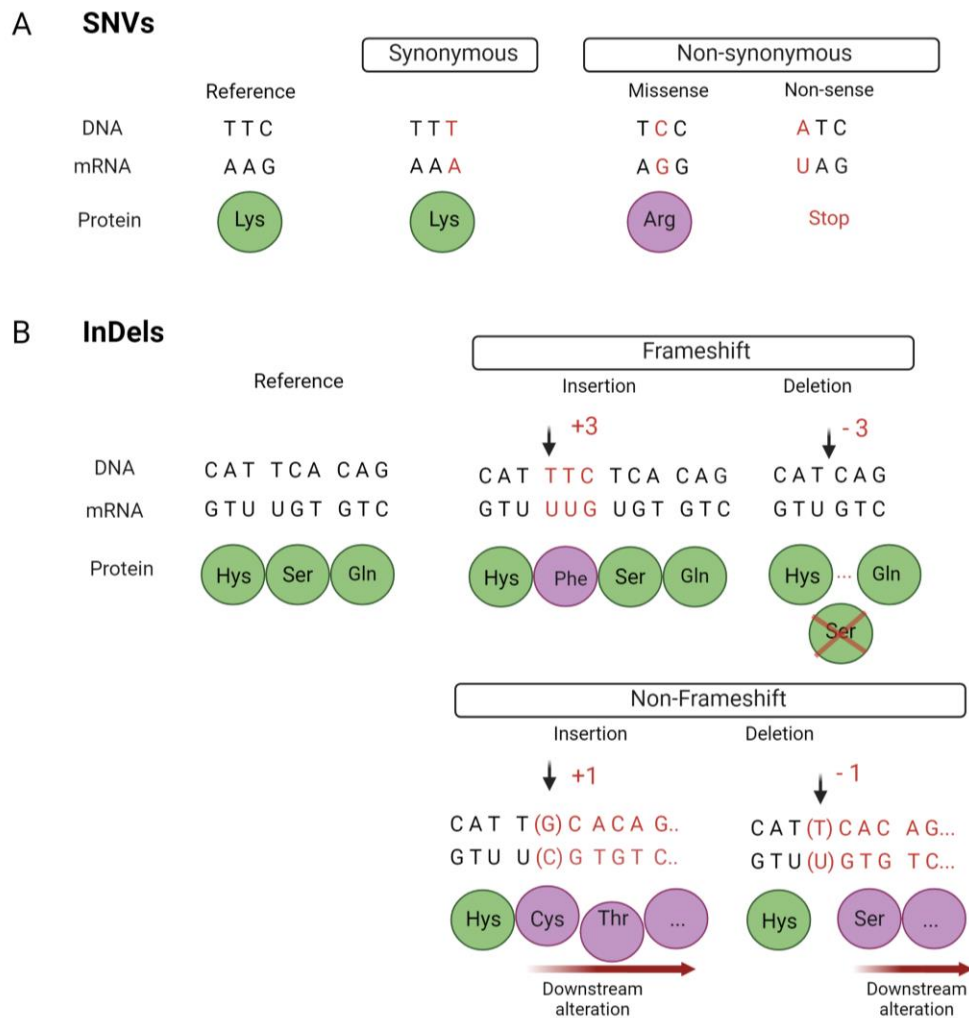
Figure 7. **Scheme of the different types of variants**. Type of genetic single nucleotide variants (A), short insertions and deletions (B). *Created with BioRender.com*.
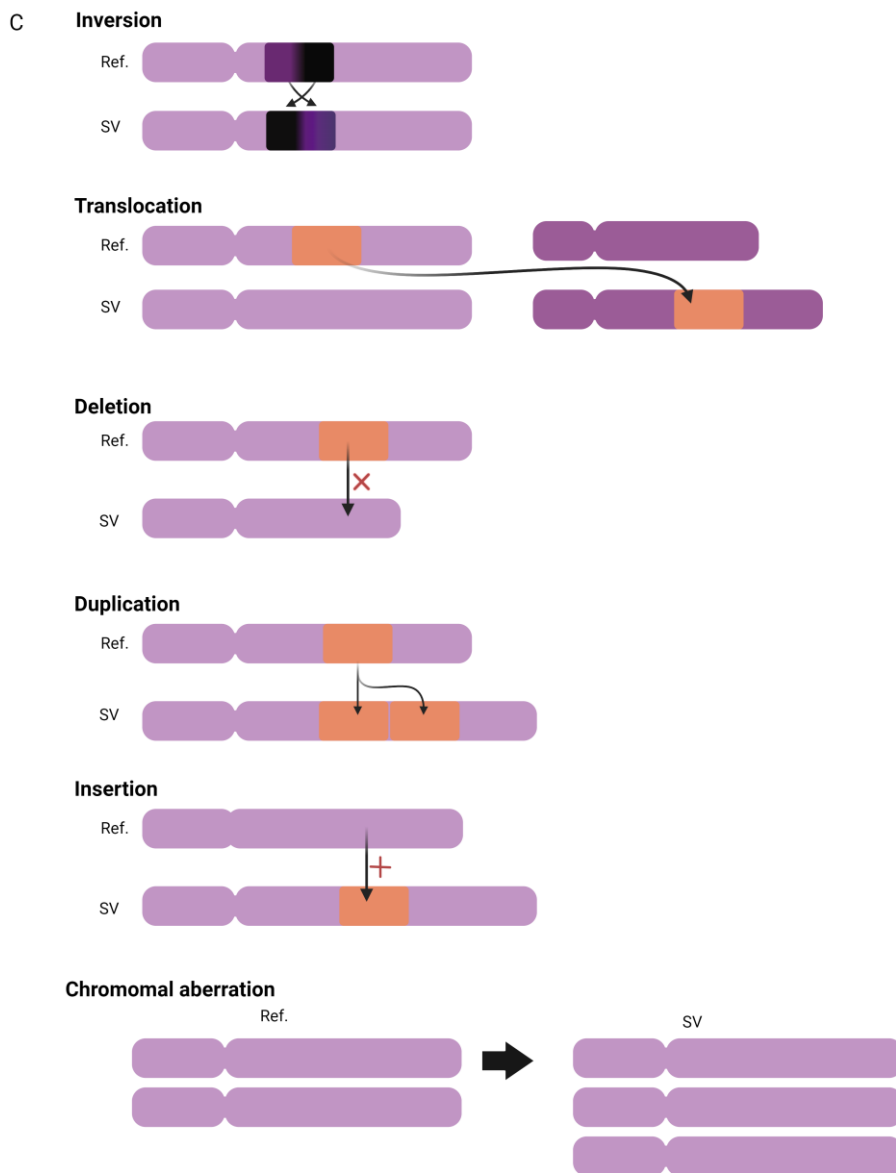
Figure 7 (continued). Scheme of the different types of variants. Structural variants (C). *Created with BioRender.com*

1.3.2 Variants annotation assessment

In genetics studies, a fundamental task is to differentiate benign and pathogenic genetic variants to prioritize them effectively for further investigation. Consequently, the emergence of predictive tools for assessing pathogenicity owes its development to extant data and technological advancements[26]. One such tool, the Combined Annotation Dependent Depletion (CADD), is designed to evaluate the deleterious potential of single nucleotide variants (SNVs) and insertion and deletion variants within the human genome. Concurrently, to ensure coherence and uniformity in outcomes across diverse tools,

guidelines for the interpretation of sequence variants have been delineated by esteemed entities such as the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)[27,28].

Additionally, variations can be evaluated based on the level of confidence in which LoF variants influence the protein. This evaluation is made easier by using the Loss-Of-Function Transcript Effect Estimator (LOFTEE)[29].

Genomic constraint denotes the selective pressure that restrains the prevalence of certain genetic variations within a population. It quantifies the evolutionary conservation of a particular genomic region or gene, indicative of its sustained stability over time owing to its vital biological function, typically associated with essential biological processes. Regions exhibiting high constraint manifest lower tolerance towards genetic variation because its variants would lead to substantial and pathogenic changes for the individual. Conversely, regions characterized by low constraint display greater tolerance towards variations, reflecting their diminished significance under selective pressure[29–31].

1.3.3 Variants analysis

Single-variant analyses (SVA) are less strong for rare variations than for common variants with comparable effect sizes; also, sample sizes should be sufficient to be useful for rare variants. Gene burden analysis (GBA) is an aggregation test that combines the frequencies of numerous genetic variants into a single score and then looks for a link between the score and a characteristic. This research strategy is effective for studying phenotypes induced by a large number of variations, as all of the effects are in the same direction[32]. Finally, a genome-wide association analysis (GWAS) is used to discover potential genetic variations that are statistically associated with a risk for a certain characteristic. GWAS are excellent for extensive cohort studies for identifying common variations[33].

1.4 Migraine

Migraine is a chronic neurovascular disorder characterized by repeated transient symptomatic episodes, affecting over one billion people worldwide [34]. It is the second most prevalent neurologic disorder after tension-type headache, with a female-to-male ratio of 3:1[35], and it has a significant economic impact.

There are several studies where this difference in prevalence is reported. According to sex, roughly 18% of adult women report experiencing migraine compared to only 6% of adult men[36]. The burden associated with migraine is underestimated even in developed countries. Costs are related to the length of the migraine. As a result, individuals with chronic migraine have major obstacles to working and performing daily activities, higher healthcare resource utilization, and worse health-related quality of life in Europe [37,38]. The financial load in the U.S. was estimated at $78 billion in 2020 [36] and the European Union €27 billion annually[39]. In Spain, some studies found that migraine's financial burden was over 1000 million euros[38,40]. Although the differences between women and men are widely remarked on, studies in twins cannot exclusively explain these variances in the prevalence with the genetic component [36].

In specific populations, the incidence of migraine sufferers is 8.1 per 1000 person-year in persons without migraine initially; however, in other populations, like Turkish, it can rise to 23.8 per 1000 person-year. The incidence rate is higher in women; the migraine onset is before 35 years old in 75% of reported cases [34]. The prevalence is estimated at 15% worldwide [35,41], highest in Southeast Asia (30%) and lowest in China (9%). Data collected from nine European countries reported a prevalence of 35%, while the USA estimation is 12% [34,41]. However, prevalence varies greatly depending on the population examined and the different geographic regions [34,42]. As a result, Nepal has the highest prevalence, attributed to living at high altitudes [43], while China has the lowest prevalence of migraine. Other factors, such as socioeconomic status, affect the prevalence of migraine other than ethnicity and geography [44,45]. We must remember that prevalence estimates between geographical regions and ethnicities are liable to methodological variations.

Migraine is the most typical neurological condition seen in primary care. It continues to lead to the second cause of disability worldwide and is the first among young women. Anamnesis and physical examination are the keys to the correct diagnosis, based on clinical criteria provided by the *International Classification of Headache Disorders, 3rd edition (ICHD-3),* published in 2018 [35,46,47].

1.4.1 Pathogenesis

Many theories have tried to explain the origin of migraine, and these theories have changed dramatically over the years [39]. Migraine has been known as far back as 3000 B.C., and evidence can be found in ancient Mesopotamian poems. However, the underlying pathogenesis still needs to be fully elucidated. The last half century has seen significant development of theories due to medical research and advancing scientific

technologies [39,43]. One of the first theories postulated by Harold Wolff in the 1940s was the existence of an underlying vascular process, which described that vasodilation of extracranial arteries and intracranial blood vessels produce mechanical activation of perivascular nerve fibers that innervate the vessels, resulting in headache pain. However, the neural theory is already obsoleted [39]. Subsequently, Ray and Wolff`s studies proposed that migraine headaches may be caused by activating nociceptive nerve fibers that innervate the meningeal blood vessels, known as vascular theory. However, it was unclear what triggered these fibers. A "Sterile neurogenic inflammation" of the dural meninges that might activate the perivascular innervation to trigger migraine was a proposal to answer this question. The activation is mediated by mediators like CGRP, substance P, neurokinin A, and prostaglandins, which increase local blood flow. However, this theory seems implausible, as not all inflammatory substances are released in migraine, and the preclinical human models do not replicate the dural plasma protein extravasation found in animal models to support this idea. These data indicate that neurogenic dural inflammation is unlikely to play a significant role in migraine origin [39].

Two aspects are the most controversial: initiation and the origin of pain. The initiation of a migraine attack is associated with internal or external triggers; although the underlying neural mechanism in people susceptible to developing migraine is unknown, it is suggested that a state of hyperexcitability exists in the brain[39].

Nowadays, it is widely accepted that the trigeminovascular system (TVS), the brainstem, and diencephalic nuclei are activated and sensitized[39]. The TVS is considered to originate and disrupt migraine pain perception[35].

The TVS involves the trigeminal nerve and blood vessels in the brain. The system involves the trigeminal nerve, which is responsible for transmitting pain signals from the meninges (the protective covering of the brain) to the brain. When the trigeminal nerve is activated, it releases neuropeptides, such as calcitonin gene-related peptide (CGRP), which cause inflammation and dilation of blood vessels in the brain. This process contributes to the pain and other symptoms associated with migraines. The TVS also incorporates several brain areas, such as the brainstem's trigeminal *nucleus caudalis*, which is involved in processing pain. The hypothalamus, essential in controlling pain perception, provides information to the trigeminal *nucleus caudalis*. It is believed that dysfunction in the TVS, such as heightened pain sensitivity or aberrant blood vessel dilatation, plays a role in the onset of migraines. It is crucial to comprehend the function of the TVS to create migraine remedies that work [39].

Only recently has knowledge broadened, and it has been understood that other elements, such as metabolic alterations, play an essential role in the genesis of migraines [43].

A simple way to explain a migraine is to split it into different phases: The premonitory phase, aura, headaches, and the postdromal phase. Not all stages necessarily have to be present in a patient, and an overlap of the different phases is possible.

a) Prodrome phase

Premonitory symptoms preceding migraine headaches have been documented for decades but are often overlooked. These symptoms, occurring up to 72 hours before the headache, include mood changes, irritability, fatigue, food cravings, yawning, stiff neck, and phonophobia. They may persist throughout the aura, headache, and postdrome phases. Imaging studies suggest the hypothalamus plays a significant role in these symptoms, as evidenced by increased blood flow during their presence. Interestingly, triggers like sleep deprivation, hunger, or bright light may represent premonitory symptoms of an ongoing attack[39].

b) Migraine aura

The aura phase of migraine is believed to be caused by cortical spreading depression, a wave of depolarization across the cerebral cortex followed by reduced blood flow. Neuroimaging shows hemodynamic changes in migraine patients with aura but not in individuals without it. Activation of the trigeminovascular system via spreading depression triggers migraine headaches. This process involves the release of inflammatory mediators that dilate intracranial arteries and sensitize trigeminal nerve fibers, leading to pain perception[35,39].

c) Headache phase

This phase is classically based on the criteria of the latest edition of the ICHD-3. It defines migraine as headache attacks lasting 4–72 hours, accompanied by nausea, photophobia, and/or phonophobia. The headache is described as unilateral, pulsating, moderate to severe intensity, and worsened by physical activity; meeting two criteria fulfills the diagnosis. The ICHD-3 also introduces a practical distinction between chronic migraine (≥15 days per month) and episodic migraine. However, the utility of this distinction, particularly the 15-day cut-off, and its contribution to physiological understanding remain unclear[39,47].

d) Migraine Postdrome

Limited studies suggest its symptoms resemble the premonitory phase, including tiredness, difficulty concentrating, and neck stiffness. It's unclear if these symptoms originate in the premonitory phase and persist through the headache phase into the postdrome phase or if they emerge during or after the headache phase. Migraine sufferers often attribute postdrome symptoms to medication that relieved their headache, suggesting they appear or reappear after the headache phase[39].

1.4.2 Diagnostic criteria

Migraine has two major types: Migraine with and without aura.

1.4.2.1 Migraine without aura

Previously known as common migraine or hemicranias simplex, migraine without aura is a clinical syndrome characterized by headaches with specific features and associated symptoms[46]. It consists of recurrent attacks lasting 4-72 hours. Typically, headache characteristics are unilateral location, pulsating quality, and moderate or severe intensity. The headache is aggravated by routine physical activity and is associated with nausea and/or photophobia and phonophobia[46]. The diagnostic criteria of migraine without aura are summarized in table 1.

Table 1. Migraine without Aura Diagnostic Criteria

Migraine without aura
1. At least five attacks that fulfill criteria 2–5
2. Headache attacks that last 4–72 hours when untreated or unsuccessfully treated
3. Headache has at least two of the following four characteristics: • Unilateral location   • Pulsating quality   • Moderate or severe pain intensity   • Aggravation by, or causing avoidance of, routine physical activity (for example, walking or climbing stairs)
4. At least one of the following during the headache: • Nausea and/or vomiting   • Photophobia and phonophobia
5. Not better accounted for by another ICHD-3 diagnosis

Notes: 1. One or a few migraine attacks may be difficult to distinguish from symptomatic migraine-like attacks. Furthermore, the nature of a single or a few attacks may be difficult to understand. Therefore, at least five attacks are required. Individuals who otherwise meet the criteria for 1.1 Migraine without aura but have had fewer than five attacks should be coded as Probable migraine without aura. 2. When the patient falls asleep during a migraine attack and wakes up without it, the duration of the attack is reckoned until the time of awakening. 3. In children and adolescents (aged under 18 years), attacks may last 2–72 hours (the evidence for untreated durations of less than two hours in children has not been substantiated).

1.4.2.2   Migraine with aura

They are also known as classical migraine, ophthalmic, hemiparaesthetic hemiplegic, or aphasic migraine. Migraine with aura is characterized by transitory focal neurological symptoms, which can accompany or precede a headache attack. Patients may experience a prodromal phase hours or days before the headaches. After the onset of the headache has passed, a postdromal phase may also manifest. These phases mostly feature symptoms like hyperactivity or hypoactivity, depression, neck pain, fatigue, or discomfort. Migraine with aura consists of recurrent attacks, lasting minutes, of unilateral, fully reversible visual, sensory, or other central nervous system symptoms that usually develop gradually and are generally followed by headaches and associated migraine symptoms. Aura is presented in one-third of migraine patients [43]. Table 2 summarizes the diagnostic criteria for migraine with aura. The aura of the migraine can be manifested with a wide variety of symptoms. Many subtypes of migraine with aura exist depending on the aura's symptoms.

Table 2. Migraine with Aura Diagnostic Criteria

Migraine with aura
A. At least two attacks that fulfill criteria 2 and 3
2. One or more of the following fully reversible aura symptoms:
• Visual
• Sensory
• Speech and/or language
• Motor
• Brainstem
• Retinal
3. At least three of the following six characteristics:
• At least one aura symptom spreads gradually over ≥5 min
• Two or more aura symptoms occur in succession
• Each individual aura symptom lasts 5–60 min
• At least one aura symptom is unilateral
• At least one aura symptom is positive
• The aura is accompanied by or followed by a headache within 60 min
4. Not better accounted for by another ICHD-3 diagnosis

Notes: 1. When, for example, three symptoms occur during an aura, the acceptable maximal duration is 3 60 minutes. Motor symptoms may last up to 72 hours. 2. Aphasia is always regarded as a unilateral symptom; dysarthria may or may not be. 3. Scintillations and pins and needles are positive symptoms of aura.

It is important to note that migraine with typical aura has a variant called typical aura with headache, characterized by an aura that is followed within 60 minutes by headaches with or without migraine-like symptoms. However, a ***typical aura without a headache*** is a migraine with a typical aura in which a headache does not follow an aura. There are

various forms of migraines other than the typical migraine with aura, such as *migraine with brainstem aura*, exhibiting aura symptoms coming from the brainstem but lacking weakening. However, *Hemiplegic migraine* includes aura with motor weakness. There is a familial variant of hemiplegic migraine (*Familial hemiplegic migraine; FHM*) in which at least one first or second-degree relative has experienced episodes fulfilling the criteria for the condition. *Retinal migraine* encompasses symptoms such as recurrent attacks of monocular vision, including scintillations, scotomata, or blindness [46].

Other primary headache diseases, mostly tension-type headaches, and certain secondary headache disorders, such as post-traumatic headaches, are included in the differential diagnosis of migraine [35]. A few topics have been modified in the ICHD-4 alfa's updated diagnostic criteria, published in 2020, although the final version has to be released [48].

1.4.3 <u>Genetics of Migraine</u>

Migraine is considered a multifactorial disorder with strong heritability and familial aggregation. Multiple genetic variants, environmental and behavioral factors exist [47]. This is supported by the difference found in the distribution of the disease by sex, which is three times greater in females. The mechanism behind migraine preponderance in women has yet to be elucidated [36]. An attempt was made to clarify the extent to which genetics and the environment influenced the differences in the distribution of migraines based on sex. It was established that genetic factors cannot fully explain the significant difference in the prevalence of migraine according to sex. The existence of environmental factors such as the masculinization of the prenatal environment, which occurs in cases where a woman has a male co-twin, due to the presence of prenatal hormones that modify the female endocrine and nervous system and can later lead to the existence of a neuroendocrine factor that favors the development of migraine in women [36]. The heritability of migraine is the same in men and women, suggesting that genetic factors contribute similarly to both sexes. In contrast, there was a slight difference in the genes underlying migraine between men and women [36]. A family history of migraine is common, with average heritability estimated at 42% [39], a range estimated from 35% to 60% [49]. Additionally, the discovery of rare variants in several genes linked to FHM, which increases the risk of severe aura symptoms, and the discovery of a genetic predisposition in family studies offer compelling evidence that migraineurs are genetically susceptible to the condition [39]. Migraine is a polygenic disease, with the rare exception of migraine-related monogenic syndromes such as FHM [35].

Overall, while the genetics of migraine is complex and not fully understood, research has identified several genes and genetic pathways that may be involved in developing the condition [39,50]. Some of these genes are potential candidate therapeutic targets like *CGRP, HTR1F, CALCA, and CALCB* [50]. Various techniques have been used to discover the different genes. One approach to studying the genetics of migraine is through genome-wide association studies (GWAS), which looks for common genetic variants associated with an increased risk of migraine [39,50]. This technique replaced poorer-performing, higher-cost candidate gene association studies (CGAS) [50]. Several GWAS have been conducted, and they have identified several genes that may be involved in migraine, including genes involved in glutamatergic neurotransmission, pain-sensing pathways, neuronal development, and brain vasculature function[39].

However, it is essential to note that GWAS has limitations and may not capture rare genetic variants or interactions between genes that may be functionally relevant. Whole exome and whole genome studies are more valuable for identifying rare variants and RNA-seq to explore the molecular mechanisms involved in migraine. In addition to GWAS, other genetic studies have looked at specific genes that may be involved in migraine. For example, mutations in the *CACNA1A, ATP1A2, SCN1A, and PRRT2* genes have been linked to FHM, a rare form inherited in an autosomal dominant pattern. Other genes implicated in migraine include the *MTHFR* gene, which is involved in folate metabolism, and the *PRDM16* gene, which is involved in neuronal development [39]. Just as a particular relationship of migraine genes has been seen with some diseases, *NCOR2* has been related to epilepsy and migraine, as has the *SLC4A4* mutation and the *PHACTR1* gene associated with migraine and Vascular diseases. [50].

It is important to note that research in this field constantly evolves, and genes and mechanisms related to migraine continue to be discovered [39]. None of the variants found is sufficient to cause the disease by itself, reaffirming that it is a polygenic disorder[50].

1.5 Tinnitus

Tinnitus is the conscious awareness of a tonal or composite noise for which there is no identifiable corresponding external acoustic source which becomes Tinnitus Disorder when associated with emotional distress, cognitive dysfunction, and/or autonomic arousal, leading to behavioural changes and functional disability. Due to the lack of agreement on the tinnitus description, this definition was proposed by the Tinnitus Research Initiative (TRI) [51]. The sounds experienced by the individuals are varied, including ringing, buzzing, whistling, whooshing, or clicking sounds. Besides, the

individual could feel the sound in one or both laterals, with different variability and pitch[51,52].

Although tinnitus can be classified according to different characteristics, they are usually described as objective and subjective tinnitus. The challenge elated to subjective tinnitus is that there are no objective tests for its assessment, so the patient's description of this symptom is the only source of information[53]. To determine if tinnitus-specific interventions are required, a validated tinnitus questionnaire can be used, along with a detailed case history and otological and audiological evaluation, to assess the level of distress related to tinnitus, the handicap related to tinnitus and the patient's emotional response to the condition. The two most frequently used questionnaires worldwide are the Tinnitus Handicap Inventory (THI) and the Tinnitus Functional Index (TFI)[54]. These questionnaires are helpful tools for quantifying the conditions' disabling and handicapping effects. They may measure adverse effects, such as difficulty sleeping, concentration difficulties, anxiety, and irritability.

1.5.1 Epidemiology

The prevalence of tinnitus shows a great variation, primarily attributed to using self-reported questionnaires in many studies for prevalence assessment. This practice poses challenges to accurate prevalence calculation. In the literature, prevalence rates for tinnitus range from 10-15%[55] to 12-30%[56]. A recent systematic literature review reported a mean prevalence of 26.3% and the white ancestry with the highest prevalence [57]. This indicates that tinnitus is a prevalent symptom in the global population. Moreover, this condition becomes an annoying symptom for up to 20% of those affected[57] and is considered severe in 1% of cases[51].

After analyzing the prevalence of tinnitus over several decades, the general conclusion is that it increases with age. The prevalence tends to increase from 4–6% in young adults to 10–20% in those aged 70–79 years[53,58].

1.5.2 Physiopathology

Despite its high prevalence, the underlying mechanisms of tinnitus remain poorly understood. Traditionally, the ear was presumed to be the only anatomical location for tinnitus. However, emerging evidence suggests that abnormal neural circuit function within the central nervous system may underlie certain forms of tinnitus [59,60].

Tinnitus is a phantom sensation of different types of sound. Phantom sensation is a perception of auditory sensations without external stimuli, wherein the aberrant neural

activity isn't strictly confined to a particular anatomical site. Instead, tinnitus involves a network comprising diverse brain structures, neurotransmitters, and receptor types. Unlike neurological disorders characterized by singular pathological structures and ensuing aberrant signaling pathways, the intricate interplay among multiple structures complicates the elucidation of tinnitus etiology[61,62].

Among the potential origins of tinnitus is its association with synaptic dysfunction occurring at the junctions between hair cells and the auditory nerve or within the auditory nerve itself. Research indicates that even minor injuries to the auditory nerve can elicit various neuronal responses within the cochlear nucleus, including heightened excitation, which can potentially be correlated with tinnitus[61,62].

Furthermore, in the auditory system, it is essential to balance things that make the nerves more active (excitatory) and things that calm them down (inhibitory). If this is altered and the inhibition is reduced, an "amplification" is produced in the neural networks, entailing self-oscillations, which could lead to some forms of tinnitus[61].

Neural plasticity emerges as a predominant factor underlying tinnitus pathogenesis[61,62]. Neural plasticity produces the nervous system's capacity to adapt its function and structure in response to both experience and injury[63]. Activation of neural plasticity may result in the unmasking of dormant synapses or the masking of normally conducting synapses, culminating in abnormal changes in connectivity and the onset of pathology. An example of change in connectivity, frequently associated with tinnitus, is the activation of non-classical sensory pathways through information rerouting, thereby facilitating cross-modal interactions.

Furthermore, the disorders in non-classical pathways may be associated with symptoms commonly concomitant with tinnitus, including hyperacusis, affective disorders, phantom phenomena, improved perceptual abilities, or aberrant sensory perceptions. Conditions of neural plasticity activation give rise to plasticity disorders such as certain types of tinnitus, phantom sensations in other sensory system, central neuropathic pain, or motor system spasms[61].

The lesions in the dorsal nucleus were examined in felines, revealing they have no reflexive responses to auditory stimuli elevation. Nonetheless, through training, these cats demonstrated the capacity to acquire responses. This observation suggests that the dorsal nucleus may serve as the initial locus of specific auditory reflex pathways, as evidenced by the absence of reflexive responses but retention of learned responses following lesions. Consequently, lesions affecting the dorsal nucleus may represent a potential etiological factor in the genesis of tinnitus[64].

1.5.3 Tinnitus and Hyperacusis

Hyperacusis is an auditory hypersensitivity disorder characterized by heightened perceptions of noise level and loudness [65,66]. Patients suffering from hyperacusis find moderate to intense sounds painful or intolerably loud [67]. The prevalence of hyperacusis is heterogeneous, ranging from 6% to 17%[68], with the large variability accounted for by age and hearing status differences. In addition, there is a suggestion that the prevalence of hyperacusis increases with advancing age and in females with higher educational levels [68].

Although many associations exist, its pathogenesis still needs to be fully understood. It may be due to acoustic overexposure, resulting in increased central auditory pathway gain [68,69].

Furthermore, hyperacusis frequently co-exists with tinnitus and can induce significant distress, with patients regularly reporting impairment in their social, occupational, and recreational activities [68]. This condition is strongly associated with tinnitus in such a way that 86% of patients with hyperacusis endorse tinnitus, and 40%-80% of patients with tinnitus endorse hyperacusis [69]. No difference between sexes was observed in the association between hyperacusis and tinnitus[70].

This correlation raises the possibility that the two disorders may be linked at some level, so disruptions in the auditory system, leading to tinnitus, may also lead to hyperacusis. While hyperacusis is almost exclusively bilateral and rarely intermittent, tinnitus is often unilateral, intermittent, and associated with somatic modulation [68].

Treatment options for hyperacusis include strategies such as avoidance of noise stimuli, tinnitus retraining therapy, cognitive behavioral therapy, and gradual sound exposure therapy. Nevertheless, there is insufficient evidence to determine the efficacy of these interventions for managing hyperacusis [69].

1.5.4 Tinnitus and Vestibular Migraine

Tinnitus and VM are closely associated. Studies have shown that tinnitus is prevalent in patients with VM, with rates ranging from 10% to 50%. The tinnitus experienced by VM patients is often unilateral and can occur during vertigo attacks or in the interval between attacks[71,72].

The mechanisms linking VM and tinnitus are not yet fully understood, but they are believed to involve the shared pathophysiology of migraine and inner ear dysfunction. Migraine-related vasospasm and neurogenic inflammation may damage the auditory system, leading to tinnitus, hearing loss, and aural fullness[73].

Differentiating tinnitus associated with VM from other causes like Meniere's disease (MD) can be challenging because the symptoms overlap significantly. Therefore, a careful clinical evaluation, including assessing migraine features and vestibular symptoms, is crucial for accurate diagnosis.

The shared triggers between VM and tinnitus suggest a potential common underlying mechanism involving neurovascular changes and inner ear dysfunction. Factors that can trigger migraine attacks, such as stress, dietary triggers, and environmental stimuli, may also contribute to the development or exacerbation of tinnitus in these patients. Understanding and avoiding these common triggers is essential in managing both VM and tinnitus. To manage tinnitus in VM patients, a combination of migraine-specific treatments, such as lifestyle modifications, preventive medications, and vestibular rehabilitation therapy, is often recommended. Addressing the underlying migraine pathology can help alleviate the associated tinnitus and other auditory symptoms[74,75].

In summary, tinnitus is a common comorbidity in patients with VM, and understanding the relationship between these conditions is essential for appropriate diagnosis and tailored treatment strategies.

1.5.5 Genetics of Tinnitus

The main goal of the scientific community studying tinnitus is to understand its origin. This is a challenge due to the variety of symptoms and related conditions. Tinnitus is known to have a multifactorial origin, including noise exposure as one of the most important environmental factor associated with its development[76]. Analyzing clinical data from adoptees revealed a significant association between the risk of experiencing tinnitus among adoptees and their biological parents, whereas no significant association was observed between adoptees and their adoptive parents. These findings indicate the involvement of genetic factors in the development of tinnitus [76]. Tinnitus has an essential effect on 15% of the families[77]. Furthermore, the risk of tinnitus recurrence in siblings was found to be greater in women than in males, with significant differences seen across genders[78]. Studies conducted on twins have shown that the heritability of bilateral tinnitus cases is significantly greater than that of unilateral tinnitus. Specifically, heritability rates were recorded at 56% for bilateral cases and 27% for unilateral cases. Moreover, there

was a notable discrepancy in heritability between genders, with men exhibiting a higher rate of 68% compared to 41% in women[79]. Another investigation involving male twin pairs determined that the relative proportion of additive genetic factors was approximately 40% of the variance[80].

In recent years, various methodologies have been employed to investigate the inheritance patterns of tinnitus. One prevalent approach in genomic analysis, commonly utilized in the study of diseases, has also been applied to tinnitus. Several GWAS have yielded diverse findings: (a) identification of an SNV in *GPM6A* along with 19 independent loci[81]; (b) discovery of an SNV in an intergenic region and another within the intron of *TNFRSF1A*[82]; (c) detection of three variants proximal to *RCOR1*[83]; (d) recognition of 17 suggestive SNVs spanning across 13 genes and a missense variant in *WDPCP*[84]. The latter two studies utilized cohorts from the UK Biobank. Through genotyping, SNVs within *GRM7, 5-HTTLPR, ADD1, BCR,* and *KCNQ1* genes were associated with tinnitus severity [85–89].

Furthermore, a mitochondrial variant was linked to tinnitus [90]. An epigenetic investigation revealed differential methylation at one CpG site for *BDNF* and three sites for *GDNF* in individuals with chronic tinnitus [91]. Selecting individuals with extreme phenotype and using gene burden analysis (GBA), an overload of rare missense variants were found in the genes *ANK2, TSC2*, and *AKAP9* in Spanish patients with tinnitus extreme phenotype [92]. These findings were replicated in Swede patients with severe tinnitus [57]. Additionally, through GBA coupled with Structural Variant analysis, candidate genes such as *CACNA1E, NAV2*, and *TMEM132D* were identified [93].

The majority of these investigations have not been replicated, showing the limitations of GWAS based on Biobank data characterized by poorly defined phenotypes concerning tinnitus, hearing loss, and other commonly associated comorbidities [94]. As an example of the complexity inherent in identifying variants associated with tinnitus via GWAS, Trpchevska et al [95]. failed to discern any variants associated with tinnitus in a large cohort with over 600,000 cases. Nonetheless, they determined 48 risk variants linked to hearing loss.

1.6 Vestibular Migraine

VM was first described in 1999 by Dieterich and Brandt and corresponds to a variant of migraine whose main symptoms are vestibular [96]. However, the first time that vertigo and headache were linked in literature was much earlier, in the XIX Century[97]. As early as

1984, Kayan and Hood reported that vestibular symptoms and other neuro-otological manifestations were more prevalent in patients with migraines than those with tension headaches [98]. In 2013, VM was recognized by the International Classification of Headache Disorders (ICHD-III), previous several terms were used for that condition, such as migrainous vertigo, migraine-related dizziness, migraine-associated vertigo, and any combination of these terms[99].

Migraine and vertigo are two very prevalent conditions in the population. The primary disorder which connects vertigo with headache is VM. Patients with VM, particularly those who experience an aura, are twice or three times more likely to develop vertigo than individuals in the general population [100].

1.6.1 Epidemiology

VM is considered the most common cause of recurrent spontaneous vertigo [101] and the second most common vestibular disorder after benign paroxysmal positional vertigo. It affects 10 to 20% of all migraine patients [99].

Migraine and vertigo are prevalent clinical conditions affecting approximately 14% and 7% of the general population, respectively. According to recent epidemiological studies, both conditions, vertigo and migraine, affect 3.2% of the population [102]. The main disorder connecting both these entities is VM, which affects approximately 1% of the general population[102–104]. However, there is literature suggesting that this prevalence may be higher, ranging from 1 to 3%[100], although, in families, it is 4 to 10 times higher[105]. The prevalence varies according to the ethnicity and country of the study population; in black ethnicities, it is 3.13%, white is 2.64%, while in Asians, it is 1.7% [105].

Although VM is one of the most frequent causes of vertigo, causing almost 10% of referrals to specialized dizziness clinics, this condition is still underdiagnosed. Therefore, it is crucial to conduct a comprehensive medical history, with particular attention to both migraine and vestibular symptoms[100].

VM may start at any age within a wide age range spanning from 19 to 79 years[96,106]. The mean age of onset in patients presenting simultaneously with vertigo and migraine is 23 years. Conversely, for patients experiencing symptoms sequentially, the mean age of onset for migraine was 24, while for vertigo, it is 37 years[105]. VM has a female preponderance of about 3:1 [99].

1.6.2 Physiopathology

The pathophysiology of VM is unknown and complex. Several factors have been identified as contributing to the disease, including genetic, neurochemical, and

inflammatory mechanisms. All of them are derived from the presumed pathophysiology of migraine. The variability of symptoms and clinical findings observed during and between attacks suggests a potential interaction between migraine and both the peripheral and central vestibular systems [99,101,104].

A spreading wave of cortical depression, often associated with cortical aura symptoms, could induce vertigo when vestibular cortex areas are affected. However, the complex nystagmus patterns observed during acute VM episodes would not be consistent with a purely cortical mechanism [102]. Many neurotransmitters implicated in migraine pathogenesis, such as calcitonin gene-related peptide, serotonin, noradrenaline, and dopamine, influence central and peripheral vestibular neurons. This suggests their potential role in the pathogenesis of VM. These neurotransmitters' diverse sites of action and their varying contributions to individual attacks may elucidate the clinical variability observed in VM. The unilateral release causes static vestibular imbalance, leading to vertigo, while bilateral release induces vestibular excitability, causing motion sickness type of dizziness[103].

The leading cause of headaches in migraine is the inflammation of intracranial vessels. Studies in mice have shown that electrical stimulation of the trigeminal nerve and intravenous serotonin administration lead to plasma extravasation in the inner ear. This mechanism could explain the vestibular and cochlear symptoms associated with inner ear dysfunction in migraine. Moreover, the observation that painful trigeminal stimulation induces nystagmus in migraine patients but not in those without migraine history suggests a heightened sensitivity in the connections between the trigeminal and vestibular systems in migraine[103,104].

Finally, another hypothesis associates VM with genetic abnormalities in ion channels, which have been implicated in various paroxysmal disorders like periodic paralysis, episodic ataxia, and FHM. Therefore, it is conceivable that defective ion channels predominantly expressed in the brain and inner ear could cause localized ion imbalances in VM, resulting in temporary dysfunction of the sensory organs within the labyrinthine and central vestibular structures. However, no such genetic defect has yet been identified in VM[99,103].

1.6.3 Clinical Features

VM shows variability in its clinical presentation [107]. Its symptoms may include spontaneous spinning vertigo, positional vertigo, as well as vertigo triggered by visual stimuli, or head motion included dizziness with nausea. These presentations may occur

in isolation, simultaneously or sequentially [99]. Some patients experience spontaneous vertigo, which is transformed into positional or head motion-induced vertigo after hours or days. In the development of the disease, up to 40-70% of patients experience positional vertigo, but not necessarily in every attack of VM [103,104].

Nausea and imbalance are frequent but nonspecific accompaniments of acute episodes of VM. Hearing loss, tinnitus, and aural pressure are the auditory symptoms more frequently related to acute attacks in 20% to 40% of patients [103]. Hearing loss associated with VM typically presents as mild and transient, with minimal progression throughout the course of the disease. Approximately 20% of individuals may experience mild bilateral downsloping sensorineural hearing loss (SNHL) over time. Almost half of patients with VM have comorbid anxiety and depression, which may cause persisting dizziness between attacks [99].

Many patients with VM experience episodes both with and without headache. Often, patients report a less severe headache during vertigo episodes compared to their typical migraine headaches. Additionally, in some cases, vertigo and headache do not occur simultaneously. Alongside vertigo, patients may also exhibit symptoms such as photophobia, phonophobia, osmophobia, and visual or other auras. These associated phenomena are diagnostically significant as they may represent the only evident link between vertigo and migraine [103]. All these vestibular symptoms often appear during headache-free intervals; only 25% of patients experience headache during vestibular attacks [104].

The temporal association of vertigo and headache varies within and between individuals[99]. VM episodes can be triggered by the same factors known to trigger migraine headaches. These include menstrual periods, irregular sleep patterns, stress, physical exertion, dehydration, and consumption of certain foods and beverages. Additionally, intense sensory stimulation can also serve as a trigger for VM episodes[106].

Vestibular symptoms typically occur several years after the disease onset, by an average of 14 years after migraine onset and 8 years after aura started, when headaches may be less frequent or absent. The onset of vestibular symptoms replacing the headache is more commonly seen in perimenopausal women[100,104,106]

Duration of episodes is highly variable; some patients need several weeks to recover fully from an attack. Only 30% of patients have episodes of minutes of duration, 30% have attacks for hours, 25% have episodes over several days, and the remaining 15% have attacks lasting seconds. The last kind of attack tends to be related to head motion,

visual stimulation, or changes in head position. In these patients, episode duration is defined as the total period during which short attacks recur[103,104].

Benign paroxysmal positional vertigo and Vestibular Migraine

VM may present with purely positional vertigo, thus mimicking Benign paroxysmal positional vertigo (BPPV), which can lead to confusion between the two conditions. Direct observation of nystagmus during the acute phase may be necessary for differentiation. In VM, positional nystagmus typically persists and exhibits constant velocity, mostly ranging from low to moderate (rarely exceeding 30°/s). Symptomatic episodes of VM tend to be shorter in duration (minutes to days rather than weeks) and more frequent. It has been observed that typical BPPV is more prevalent in patients with migraine, although the reasons for this association remain unclear[108].

Chronic Vestibular Migraine

Although VM is typically conceptualized as an episodic vestibular disorder, reports of a chronic variant of VM have been published. Many patients may experience varying degrees of visually-induced, head motion-induced, or persistent dizziness between episodes. Distinguishing between chronic VM, motion sickness, and comorbid persistent postural-perceptual dizziness poses a notable challenge in these individuals[108].

1.6.4 Diagnosis

1.6.4.1 Physical examination

In most patients with VM, neurologic and otologic examination is normal between crises. The neuro-ophthalmologic evaluation may reveal mild central deficits such as persistent positional nystagmus and saccadic posit, particularly in patients with a long history of VM[103]. Although nystagmus with peripheral involvement characteristics can be found too[106]. Intercritical head-shaking nystagmus occurs in less than 50% of patients[99].

During the acute phase of VM, most patients exhibit central spontaneous nystagmus, central positional nystagmus, or a combination of both. The most common is a central type of spontaneous nystagmus such as downbeat, upbeat, or torsional nystagmus[99]. Positional nystagmus typically persists and often manifests as horizontal nystagmus, beating either towards the lower ear (geotropic nystagmus) or towards the upper ear (apogeotropic nystagmus). Patients may sometimes present with a peripheral type of spontaneous nystagmus and a unilateral deficit of the horizontal vestibulo-ocular reflex.

Imbalance is commonly reported during acute attacks, while transient mild to moderate hearing loss is not common[103].

1.6.4.2   Diagnosis criteria

The International Headache Society and the Barany Society, representing the international neurotological community, collaborated in 2012 to publish the first consensus on diagnostic criteria for VM[109], which have been revised in 2022.[108]

Diagnostic criteria for VM were proposed by Neuhauser in 2001 and revised in 2012 by the Bárány Society, together with the International Headache Society (IHS), which included it in an appendix in 2013 of the ICHD-3 as the first step to identifying new entities [106]. Therefore, the diagnosis of VM is based on the patient's history and the fulfillment of these criteria[109] (Table 3).

Table 3. Diagnostic Criteria of Vestibular Migraine

Vestibular Migraine
A. At least 5 episodes with vestibular symptoms of moderate or severe intensity, lasting 5 minutes to 72 hours
B. Current or previous history of migraine with or without aura according to the International Classification of Headache Disorders (ICHD-3)
C. One or more migraine features with at least 50% of the vestibular episodes: • Headache with at least 2 of the following characteristics: 1-sided location, pulsating quality, moderate or severe pain intensity, aggravation by routine physical activity. • Photophobia and phonophobia • Visual aura
D. Not better accounted for by another vestibular or ICHD diagnosis

These criteria define VM as the types of vestibular symptoms, minimum number, severity, and duration of crisis; association of vestibular and migrainous symptoms; requirement for a migraine diagnosis and absence of other causes of symptoms[104].

A separate diagnostic category of *probable* VM can prove useful for patients who do not fully the above criteria but are still considered to have VM as the most probable diagnosis[99] (Table 4).

Table 4. Diagnostic Criteria of Probable Vestibular Migraine

Probable Vestibular Migraine
A. At least 5 episodes with vestibular symptoms of moderate or severe intensity, lasting 5 minutes to 72 hours
B. Only 1 of the criteria B and C for vestibular migraine is fulfilled (migraine history or migraine features during the episode)
C. Not better accounted for by another vestibular or ICHD diagnosis

1.6.4.3   Vestibular tests

VM has an eminently clinical diagnosis based on symptoms and clinical history; there is no specific testing abnormality in VM. However, numerous vestibular abnormalities have been reported in patients with VM. Nevertheless, no specific vestibular examination is currently employed to define VM. There are discrepancies in the results of vestibular examinations of VM[110].

Video head impulse test (v-HIT) is one of the latest tools to evaluate the vestibular function, measuring its vestibular-ocular reflex (VOR) gain[111]. However, the v-HIT has not demonstrated significant alterations when compared to healthy individuals, either during acute attacks[112] or in the intercrisis period[111]Some differences between the VOR gains could be found, but these are inconsistent across in the different studies. V-HIT is useful in evaluating the response to medical treatment and in differential diagnosis of VM and MD [112].

Vestibular-evoked myogenic potential (VEMP) testing allows the electrophysiological recording of the inhibitory muscle reflex produced by the saccullo-collic pathway as a result of auditory stimuli[113]. However, their utilization is not widely adopted for the diagnosis of VM due to current literature findings indicating their inability to diagnose VM definitively[113]. Nonetheless, they do offer some utility in the differential diagnosis between VM and MD[114].

Oculomotor abnormalities are commonly seen; the most frequent findings are abnormal saccade test, abnormal smooth pursuit, and central positional nystagmus, such as downbeat, which can be observed in videonystagmography. These findings are especially notable during acute attacks[101]. While caloric tests are very variable and they are normal in most cases[115].

Therefore, while vestibular tests can assist in diagnosis, none of these tests alone serves as a diagnostic tool for VM.

1.6.4.4   Neuroimaging in VM

Neuroimaging tests, mainly Computed Tomography (CT) scan and magnetic resonance imaging (MRI), offer valuable insights into diagnosing and identifying VM. The changes observed in neuroimaging tests serve as potential markers distinguishing VM from other vestibular disorders.

The usefulness of neuroimaging in VM resides in identifying structural changes; mainly, studies have shown that there are widespread cortical structural abnormalities in the brain regions related to pain and sensory processing in patients with VM. The most recent insight shows GM volume of nociceptive and multisensory vestibular areas are increased in patients with VM compared to controls[116]. On the contrary, previous studies showed a decrease of gray matter volume bilaterally in the inferior temporal gyrus, the cingulate cortex, and the posterior insula. A reduction in GM volume was additionally noted in the left superior temporal gyrus, middle temporal gyrus, supramarginal gyrus, and superior parietal lobules. Similarly, reductions were observed on the right side of the dorsolateral prefrontal cortex and the inferior occipital gyrus. These findings suggested that these areas are involved in cortical processing of vestibular and nociceptive information[117]. Although these events are not in all patients with VM, there is a high percentage of patients with VM who have a normal MRI[118].

Neuroimaging with CT is more useful for ruling out other potential causes of dizziness and vertigo rather than directly confirming a diagnosis of VM.

To summarize, although neuroimaging can offer valuable insights into the structural and functional alterations within the brain linked to VM, its principal role lies in elucidating the condition's pathophysiology rather than serving as a standard diagnostic tool. Imaging is usually not required when a patient reports a long history of VM according to the above criteria with complete remission of symptoms during the interval[99].

1.7 Genetics of Vestibular Migraine

VM has been associated with a genetic predisposition; its understanding can contribute to elucidating its pathophysiology. There is epidemiological evidence supporting a genetic contribution to VM[105].

The early and simultaneous onset of symptoms observed in familial VM suggests genetic anticipation, a phenomenon characterized by the progression of severity of an inherited disorder in successive generations. This phenomenon is frequently encountered in neurological disorders and associated with the expansion of nucleotide repeats reported

a significantly lower age of migraine onset in patients with a familial history of migraine or VM compared to those without such a history[105].

Although no specific inheritance patterns or genetic alterations cause VM, there is likely some degree of heritability that is supported by the familial aggregation observed in some studies[119]. This is also reinforced by family history, as indicated by a large prospective series where 70% of patients reported a family history of migraine and 66% reported a family history of vertigo[105].

Therefore, a strong genetic component in VM can be assumed, similar to common forms of migraine with a polygenic basis, with at least 47 loci affecting the susceptibility. Familial occurrence in VM supports the hypothesis of heritability with an autosomal inheritance pattern with incomplete penetrance[120]. Familial VM has been linked to chromosomes 22q12, 11q, and 5q35, but no specific genetic mutations have been identified[103,120].

Currently, only one study identifies a potential candidate gene, *TRPM7*, for VM. This study focused on a Korean family with multiple members affected[121]VM has also been correlated with the *5-HRT6* polymorphism and the allelic variant of *PRG*, but further studies are needed to validate these results.[122,123]. Genes such as *CACNA1A, SCN1A*, and ATP1A2, which induce alterations in ionic channels and result in a disruption of cellular homeostasis, are known to be associated with FHM. These genes are suspected to play a role in susceptibility to other migraine syndromes, including VM. However, no direct link between these genes and VM has been established[104,120,124].

2 HYPOTHESIS AND AIMS

2.1 *Hypothesis*

The underlying mechanism of VM has yet to be elucidated, and it is believed to be due to the parallel activation of vestibular and cranial nociceptive pathways. This may be a common determinant for developing tinnitus and hyperacusis in these patients.

Our hypothesis suggests that there are specific psychoacoustic characteristics in patients with VM and a familial hereditary component that has not been described to date.

2.2 *Aims*

This Doctoral Thesis aims to accurately identify patients with VM and tinnitus and determine if there is a relationship between both entities to study the relationship between hyperacusis and tinnitus in patients with VM. Furthermore, we will identify the existence of a genetic component through the study of families with multiple cases of VM.

2.2.1 Specific aims

1) To comprehensively analyze the clinical-demographic variables in hospital-based tinnitus patients. To define patient subgroups and to identify predictors for their classification.

2) To characterize the clinical and psychoacoustic features of patients with tinnitus and VM.

3) To perform a whole exome sequencing (WES) analysis in 4 multi-case families of VM to identify variants segregating the phenotype.

3 MATERIALS AND METHODS

3.1 Patients Cohorts

Different cohorts of patients were employed to conduct various analyses to achieve the aims of this doctoral thesis. Thus, each cohort was used for each of the specific aims.

3.1.1 <u>Tinnitus cohort</u>

For the first aim, we compared the findings obtained using a self-reported questionnaire (European School for Interdisciplinary Inventory Screening Questionnaire- ESIT-SQ) completed by patients attending the Ear Nose Throat (ENT) outpatient clinics to those received online. (Annex 1)

The sample consisted of 434 patients with chronic tinnitus (>6 months duration). The first group consisted of 204 outpatients who visited the ENT departments at Hospital Universitario Clínico San Cecilio and Hospital Universitario Virgen de las Nieves from Granada (Spain). The second group included 230 individuals recruited through an online survey promoted on the hospital website and social media (https://www.genyo.es/).

<u>Inclusion Criteria</u>

The inclusion criteria for all participants were:

- Adult individuals (over 18 years old)
- Tinnitus history for at least six months, regardless of the hearing loss threshold.

The exclusion criteria were:

- To suffer a major disease that could influence the responses in the ESIT-SQ.
- Acute psychotic illness.
- Addiction disorder.
- Acute ontological disease.
- Chronic otitis.
- Vertigo crisis.
- Any other condition apart from tinnitus itself.
- Individuals unable to understand Spanish

<u>Clinical Data</u>

To analyze the variables using the ESIT-SQ, we constructed three sets of variables for data management: sociodemographic data, comorbid conditions, and tinnitus characteristics.

The sociodemographic data group included age, sex, height, weight, study level, smoking habits, and familiar members with tinnitus and dizziness. The WHO classification for the body mass index (underweight, average weight, overweight, and obesity) was used to classify the patients. To aid data analysis and interpretation, the variables hyperacusis, family history of tinnitus in first-degree relatives, and tinnitus were categorized. So hyperacusis and tinnitus were converted into dichotomous variables (yes/no), and a family history of tinnitus in first-degree relatives was classified as no/three or less/more than three).

The comorbid condition included frequency of dizziness, otological disease, surgical history, hearing loss, hyperacusis, audiological devices, pain (including headache, neck pain, and otalgia), and medical history (neurological disorder, sleep disorder, heart conditions, metabolic disorders, and psychiatric disorder especially).

Finally, the *tinnitus characterization* included tinnitus frequency, duration from the onset, the time the patients perceive the tinnitus as annoying, the patient's concern about tinnitus, the number of different sounds (one or more than one), types of onset (gradual or sudden), identifiable triggers, rhythmicity, the presence of aggravating and attenuating factors, and previous medical care and treatments used by the patients.

3.1.2 Vestibular Migraine cohort

To complete the study's second aim, 51 individuals with definite or probable VM were enrolled according to the Classification of the International Headache Society and the Barany Society diagnostic criteria. These patients were diagnosed at the Virgen de las Nieves University Hospital, Baza Hospital, and Ospedale Maggiore di Milano outpatient clinics.

Selection Criteria

The inclusion criteria for all participants were:

- Adult individuals (over 18 years old)
- European origin
- Patients with a diagnosis of definitive or probable VM

The exclusion criteria were:

- Patients with a personal history of otological disease
- Patients with a diagnosis of definite or probable MD
- Different ancestry from European

This sample of 51 individuals with VM was split into two categories. The first subgroup comprised 38 patients with VM and tinnitus, accounting for 76% of the total, whereas the second category included the remaining 13 individuals (24%).

3.1.3 VM familial cohort

Four VM families with multiple cases were recruited to search for rare variants. We obtained saliva samples from 19 individuals within all families.

3.1.3.1   Family 1

The first family from Granada spans three generations (Figure 8). In the first generation, there is a female with probable VM, I-1. In the second generation, there are two females with definite VM and complete phenotype, II-1 and II-2. In the third generation, there is a male with migraine but without vestibular symptoms, III-3.

All individuals were asymptomatic at the time of the medical interview.
Unfortunately, we have no information about the rest of the individuals in this family.

Figure 8. VM Family 1 Pedigree. Black-filled symbols: definite VM; Black and white-filled symbols: probable VM; white-filled symbols: healthy controls; oblique-lined filled symbols: individuals with migraine without vestibular symptoms; strikethrough symbols: deceased individuals; dotted line symbols: individuals where it was not possible to collect sample or clinical information; arrow point to proband.

3.1.3.2   Family 2

The second family, also from Granada, consisted of three generations with multiple cases in generations II and III. The main study subject has three sisters and five brothers. The male siblings were asymptomatic for VM symptoms, whereas all the female siblings exhibited either the complete or incomplete phenotype of the disease. As can be seen in the pedigree diagram (Figure 9), there are several individuals for whom we lack clinical information. Additionally, some subjects met the criteria for VM and probable VM, but

unfortunately, we could not obtain information questionnaires or saliva samples from them (subjects I-1, III-3, and III-6). However, we have obtained clinical information from the anamnesis.

Subject I-1 is an 89-year-old woman who has experienced migraine headaches since her youth. These migraines decreased in intensity and frequency with menopause. There is no known history of vestibular symptoms. Subject III-3 is a 24-year-old woman with an incomplete VM phenotype. Subject III-6 is a 24-year-old woman with a complete VM phenotype.
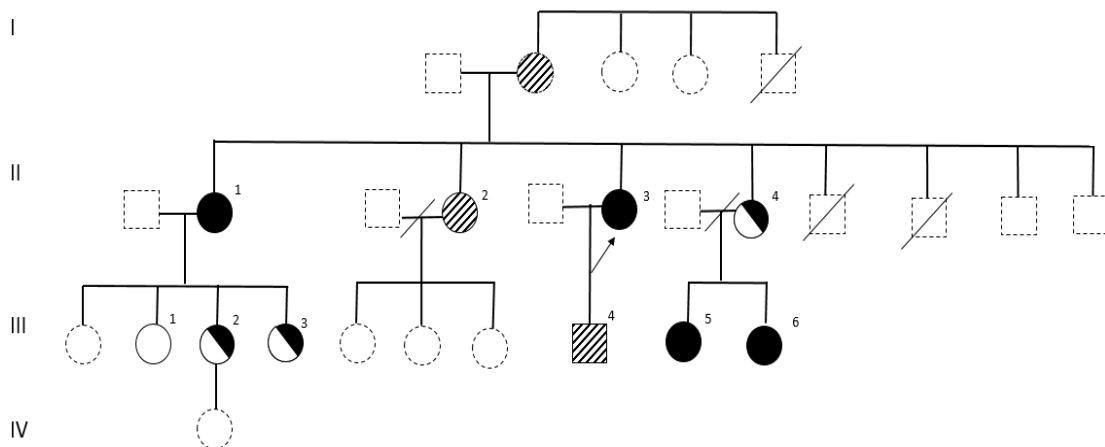
Figure 9. VM Family 2 Pedigree. Black-filled symbols: definite VM; Black and white-filled symbols: probable VM; white-filled symbols: healthy controls; oblique lined filled symbols: individuals with migraine without vestibular symptoms; strikethrough symbols: deceased individuals; dotted line symbols: individuals where it was not possible to collect sample or clinical information; arrow point to proband; strikethrough line point to relationships between individuals that no longer continue.

3.1.3.3   Family 3

The third family, originating from Italy, comprises a woman with a complete phenotype, II-1, her father who also exhibits a complete phenotype I-1, and a daughter, III-1, with an incomplete phenotype (Figure 10). Unfortunately, the clinical history of the remaining family members is unknown, although there are reports of several cases of migraine and associated vertigo. Additionally, all three study subjects and the rest of the family have a history of autoimmune thyroiditis.
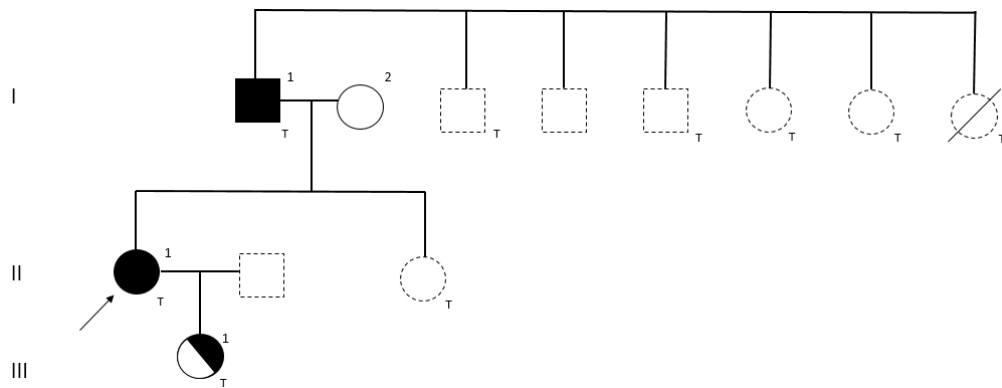
Figure 10. VM Family 3 Pedigree. Black-filled symbols: definite VM; Black and white-filled symbols: probable VM; white-filled symbols: healthy controls; oblique lined filled symbols: individuals with migraine without vestibular symptoms; strikethrough symbols: deceased individuals; dotted line symbols: individuals where it was not possible to collect sample or clinical information; arrow points the proband. T: points affected by Hashimoto's thyroiditis.

3.1.3.4   Family 4

The final family, originally from Italy, consists of the proband III-1, who exhibits VM, the mother, II-1, who also displays the complete phenotype of VM, and the proband's sister, III-2, who has probable VM (Figure 11).

Unfortunately, clinical information regarding the proband's father or maternal grandfather was unavailable. However, there are reports of migraine history concerning the maternal grandmother.

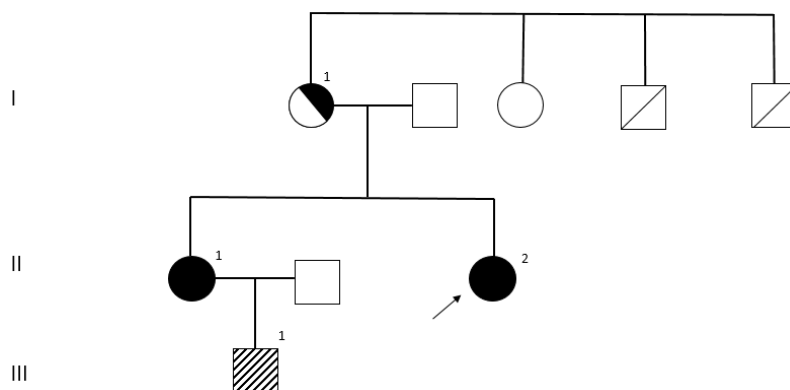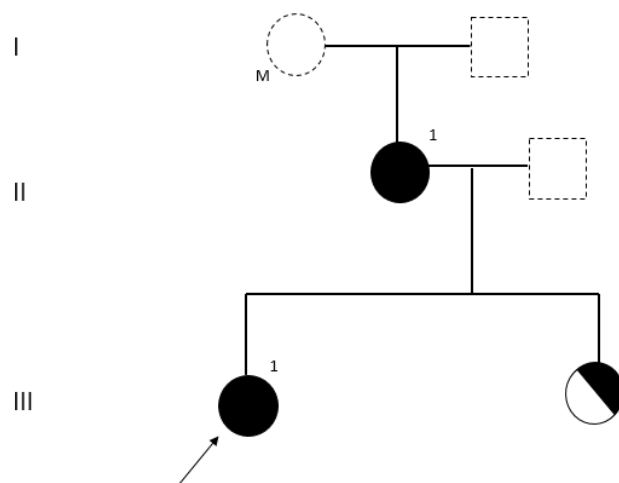**Figure 11.** Pedigree of family 4 of VM. Black-filled symbols: definite VM; Black and white-filled symbols: probable VM; white-filled symbols: healthy controls; oblique lined filled symbols: individuals with migraine without vestibular symptoms; strikethrough symbols: deceased individuals; dotted line symbols: individuals where it was not possible to collect sample or clinical information; arrow points the proband.

3.2 Audiological Assessment

3.2.1 Pure Tone Audiometry

A hearing test was conducted using pure-tone air-conduction audiometry, covering frequencies from 125 to 8000 Hz, and Pure-Tone-High-frequency audiogram ranging from 9000 to 20000 Hz. Bone conduction was assessed at 250, 500, 1000, 2000, and 4000 Hz frequencies. The Pure Tone Average (PTA) was calculated to determine the average hearing level in each patient. All audiology tests occurred in a soundproof booth, employing the Interacoustics AC40 clinical audiometer (Middelfart, Denmark)[125,126].

Each patient received brief instructions to understand the testing process. Those with tinnitus were instructed to disregard their tinnitus during the test, focusing only on responding to the test tones to determine their hearing thresholds. The patient's response to each test tone was recorded using a response button connected to a signal light. The evaluation for each frequency began at an audible level based on the patient's age. After an initial positive response, the tester decreased the tone in 10 decibels hearing level (dbHL) steps until no further response was observed. If there was no response, the evaluator increased the tone level in 5 dbHL steps until a response occurred. Following the initial response using an ascending approach, the stimulus was reduced by ten dbHL, initiating another ascending series of 5 dbHL steps until the subject responded. This procedure was repeated until the subject responded at the same level in at least half of the series, involving two, three, or four responses (i.e., 50% or more) on the ascent, determining this as the hearing threshold level. The threshold is the lowest level at which responses occur in at least half of a series of ascending trials, with a minimum of two responses required at that intensity[127].

3.2.2 Psychoacoustic Characterization and Acuphenometry

An online tool was used to identify a tone that matches the perceived tinnitus sound to assess tinnitus frequency and loudness (http://www.onlinetonegenerator.com). The procedure was initiated by introducing pure-tone sounds at a baseline frequency of 1000 Hz. Based on patient feedback, the frequency was adjusted upward or downward until the tone most closely resembling their tinnitus was identified within a frequency range of 125-16000Hz. In cases where the patient's tinnitus did not align with a pure tone, various types of noise (white, brown, or pink) were presented, allowing the patient to determine the closest match.

Subsequently, acuphenometry was conducted in a soundproof room to validate the nature of the identified noise or frequency from the tone generator. This process involved

measuring the loudness of the tinnitus (in dB SL) and determining the minimum masking level (MML). Additionally, any observed residual inhibition in the affected ear was documented, with positive inhibition recorded when the intensity of tinnitus decreased or disappeared entirely for at least 20 seconds.

3.3 Psychometric Characterization. Tinnitus

The Tinnitus Handicap Inventory (THI) questionnaire was applied to assess tinnitus-related discomfort and measure tinnitus' impact on the patient's quality of life[128]. In addition, we used the Hospital Anxiety and Depression Scale (HADS), a standardized depression and anxiety questionnaire related to tinnitus, and the European School for Interdisciplinary Inventory Screening Questionnaire (ESIT-SQ), a self-reported tinnitus-relevant history questionnaire.

3.3.1 THI

All individuals answered the THI Spanish-validated test. It evaluates the annoyance related to tinnitus and establishes the subscales of functional, emotional, and catastrophizing tinnitus. The questionnaire includes 25 questions (Annex 2), which could be answered as: "yes" (4 points), "sometimes" (2 points), or "no" (0 points). The total score ranges from 0 to 100 and classifies the individuals into five levels of severity (Table 5) [129,130].

Table 5. Classification according to the THI score

THI score	Tinnitus handicap	Grade
0-16	Slight or none	I
18-36	Mild	II
38-56	Moderate	III
58-76	Severe	IV
78-100	Catastrophic	V

3.3.2 HADS

The participants answered the Spanish version of the HADS questionnaire, consisting of two subscales: anxiety (HADS-A) and depression (HADS-D), each with seven items (Annex 3). The possible responses are rated from 0 to 3. Therefore, the total scores for each state range from 0 to 21 (Table 6) [131,132].

Table 6. Classification according to HADS-A and HADS-D scores

HADS-A or HADS-D score	State of anxiety or depression
0-7	Normal
8-10	Borderline abnormal (borderline case)
11-21	Abnormal (case)

3.3.3 ESIT-SQ

The ESIT-SQ consists of 39 closed, mainly multiple-choice questions structured in two parts. Part A includes 17 questions that everyone can answer regardless of whether they have tinnitus. The last of these questions screens for tinnitus's presence lasting more than five minutes over the past year. Participants who respond 'yes' to this question are prompted to answer 22 more tinnitus-related questions in Part B. An ENT specialist evaluated the patients, and they completed the questionnaire as part of the routine clinical practice. This questionnaire was designed by a multidisciplinary panel of epidemiologists, psychologists, and ENT specialists from ESIT to obtain a comprehensive assessment of tinnitus, other comorbid conditions, quality of life, sociodemographic data, and tinnitus characteristics [133].

3.4 Psychometric Characterization. Hyperacusis

3.4.1 Questionnaire hyperacusis sound- *Geräuschüberempfindlichkeit test*

All individuals were asked to answer the Spanish version of the *Geräuschüberempfindlichkeit test* (GÜF; also known as the Nelting test) to assess their hyperacusis. It consists of 15 questions (Annex 4) with the options: "never" (0 points), "sometimes" (1 point), "frequently" (2 points) and "always" (3 points). So, the total score ranges between 0 and 45, and the individuals may be organized into four handicap grades, described in Table 7 [134,135].

Table 7. Classification according to the hypersensitivity to sound - GÜF

Nelting score	Hyperacusis handicap	Grade
0-10	Slight handicap	I
11-17	Moderate handicap	II
18-25	Severe handicap	III
26-45	Very severe handicap	IV

3.4.2 Khalfa test

The Khalfa sound hypersensitivity test is a clinical assessment used to measure extreme auditory sensitivity or intolerance to sounds in individuals experiencing issues with sound hypersensitivity. It was developed by Dr. Salah Khalfa and it consists of two parts[136] (Annex 5).

The first part includes three binary questions providing general information on auditory disorders and noise exposure.

The second part comprises 14 self-rating items that are scored on a 4-point scale, ranging from 'no' (0 points) to 'yes, a lot' (3 points). The scores greater than 28 on the questionnaire is considered a cut-off point for identifying individuals with hyperacusis[136]. However, some authors have considered the cut-off point of 28 to be too high and have proposed a cut-off point of 16 to determine hyperacusis. This adjustment aims to improve the sensitivity of the questionnaire in identifying individuals with hyperacusis, ensuring that those with milder symptoms are not overlooked[137].

The test is designed to assess three dimensions or subscale related to hyperacusis; cognitive behavior, somatic behavior and emotional behavior[136].

3.5   Statistical Analysis

All statistical analyses were performed using SPSS v25.0 (IBM Corporation, Armonk, NY, USA). The ordinal variables were treated as continuous variables in the study, while categorical variables were coded as dummy variables. The p-values were adjusted for multiple testing using the Bonferroni correction, and a significant level of $p \leq 0.05$ was used for all statistical tests.

3.5.1 Tinnitus Cohort

First, we performed an exploratory statistical analysis for each group of participants (outpatients and online groups) separately. Since both groups had significant differences in sex and age distribution, we adjusted these variables to compare both groups in a second set of analyses. Since the online survey was distributed among members of the Spanish Association of Patients with MD ("Asociación Síndrome de Meniere España", ASMES), many respondents reported a diagnosis of MD. Because of this bias, a stratified analysis was also performed to compare the tinnitus profile in patients with or without MD.

Differences between groups were analyzed by contingency tables using t-tests and χ^2-tests for independent samples, including odds ratio with 95% confidence interval. Quantitative variables following normal distribution were expressed in mean $+/-$ standard deviations (SD). On the other hand, variables not following normal distribution were summarized through medians and interquartile ranks (25–75%). Qualitative variables were summarized through absolute and relative frequencies.

3.5.2 VM Cohort

Statistical analysis was conducted to investigate the differences between groups. The study was performed using contingency tables and t-tests. The U Mann-Whitney test was used for independent samples, and an odds ratio with a 95% confidence interval was included. Variables that did not follow a normal distribution were summarized using medians and interquartile ranks (25-75%). Qualitative variables were summarized using absolute and relative frequencies.

The Spearman correlation test was used to study the correlation between variables, as the variables did not follow a normal distribution. Linear regression was used to determine the association between the different psychometric variables. A multiple linear regression was conducted to determine which variables influenced the presence of tinnitus in VM.

3.6 Age Clustering

Clustering is an unsupervised machine learning (ML) technique aiming to group similar data points in a dataset. To use this technique, a human expert specifies the number of expected clusters or groups, which the algorithm discovers by assigning each data point to the nearest cluster center. We used the K-Means cluster algorithm, randomly selecting K points as the centers for each K cluster. After assigning each data point to the closest cluster, the algorithm updates the cluster centers by computing the average of all the points assigned to that cluster. This process is repeated until the data assigned to the clusters stabilizes. In our study, we only used clustering to partition patients into two groups based on age, young and old, so that we could investigate variables that differ between online and outpatient cohorts among people of similar ages. To achieve this, we used the clustering technique to group patients by age, where a 45-year-old patient would be closer to a cluster centered at age 25 than to a cluster centered at age 75. As the algorithm updates the cluster centers, some data points may change their allegiance to different clusters, causing further changes to the cluster centers in the next step. In our approach, responses from outpatients and the online survey were merged and

clustered into two groups (young and old individuals) following the k-means algorithm. Online and outpatient responses were compared for each variable in young and old individuals. A significant p-value for an odds ratio (OR) < 1 means outpatients reported this variable more frequently. The significance threshold for the p-value after the Bonferroni correction was <0.0026.

3.7 Exome Sequencing

3.7.1 Reagents

The following list includes the molecular biology reagents used for sample preservation, DNA extraction, WES libraries preparation, sequencing, and quality controls (electrophoresis and concentration measurements).

- Oragene®DNA (#OG-510, Genotek, Ottawa, Canada)
- prepIT L2P (#PT-2LP, Genotek, Ottawa, Canada)
- Molecular biology grade Water (#7732-18-5, Sigma-Aldrich)
- Qubit dsDNA BR Assay Kit (#Q32850, Invitrogen)
- AmpliTaq 360 DNA polymerase (#4398886, ThermoFisher Scientific)
- Agarose routine grade (#MB14403, NZYtech)
- Tris-acetate-EDTA buffer (TAE) (#B49, ThermoFisher Scientific)
- Gelred Nucleic Acid Gel Stain 10.000x in water 1*0.5 ml (Biotium, #41003)
- Loading buffer (#10816015, Invitrogen, ThermoFisher)
- Direct Load 1 Kb DNA ladder (#D3937, Sigma-Aldrich)
- 100bp DNA ladder (#15628019, Invitrogen)
- SureSelectXT Human All Exon V6 (Agilent Technologies)
- TapeStation DNA screenTape D1000 (Agilent Technologies)
- MicroElute® Cycle Pure Kit (#D6293-01, Omega, BioTek)

3.7.2 Equipment

- Nanodrop 2000C Spectrophotometer (ThermoFisher Scientific)
- Nanodrop2000 v1.4.1 (ThermoFisher Scientific)
- MicroStart 17R microcentrifuge (VWR)
- 5804 R Centrifuge (Eppendorf)
- Dry Bath/Block (#88870001, ThermoFisher Scientific)
- NovaSeq 6000 platform (Illumina)
- Workstation: AMD Threadripper RX with 32 cores and 128 GB RAM.
- Server: NAS Synology Rack (3 U) RS3617xs with 100TB of storage.

3.7.3 Informatic Resources

- ClinVar - https://www.clinicalgenome.org/
- DisGeNet - https://www.disgenet.org/
- gEAR portal - https://umgear.org/
- Gene Ontology (GO) - http://geneontology.org/
- GTEX - https://www.gtexportal.org/
- Hereditary Hearing Loss Homepage - https://hereditaryhearingloss.org/
- HPO - https://hpo.jax.org/app/
- KEGG - https://www.genome.jp/kegg/
- MGI - https://www.informatics.jax.org/
- OMIM - https://www.omim.org/
- Reactome - https://reactome.org/
- SAMTOOLS - https://samtools.github.io
- Uniprot - https://www.uniprot.org/

3.7.4 Sample Collection, DNA Isolation, and Quality Controls

Saliva samples were obtained from patients using the Oragene®DNA kit. DNA extraction was performed using the preIT®.L2P kit following the manufacturer's instructions. To ensure the quality of the genomic DNA, we conducted concentration and quality controls using Nanodrop 2000C and Qubit (dsDNA BR Assay). Additionally, to assess the integrity of the gDNA, samples and loading buffer were prepared in (1:4 volume) and loaded in 2% agarose gel in 1X TAE buffer marked with GelRed as staining agent and using Direct Load 1kb DNA as molecular weight marker. The electrophoresis was run at 90V using a PowerPac supply for 1 hour and revealed in ImageQuant LAS 4000.

3.7.5 WES library preparation and sequencing

SureSelectXT Human All Exon V6 was used to prepare the libraries targeting coding regions, following manufacturer instructions[138]. Briefly, gDNA is fragmented, denatured, and hybridized with biotin-conjugated oligos targeting coding regions. Streptavidin-paramagnetic beads purify biotin-captured fragments that are subsequently amplified and indexed by PCR. This procedure targets around 50Mb of human exonic regions. The quality of the libraries was confirmed using the TapeStation DNA screenTape D1000. Sequencing was performed on a NovaSeq 6000 platform and generated pair-end reads, ensuring a minimum coverage of 100X on average (Macrogen, South Korea).

3.8 Bioinformatics Analysis

The bioinformatics processing of WES data has several steps: 1) preprocessing, including alignment of reads with reference genome and filtering of low-quality mapped reads; 2) variant calling, identifying changes differing between samples and reference genome; 3) annotation, retrieving information of variant in public databases; and 4) data analysis, in order to discover candidate variants and genes. We followed the nf-core/sarek nextflow pipeline v3.4.2 as a workflow for the alignment and variant calling for SNV and shorts Indels[139].

3.8.1 Data Pre-processing and Alignment

Paired-end FASTQ files from each sample were generated after sequencing. Then, these sequences were aligned to the GRCh38/hg38 reference genome using the Burrows-Wheeler Aligner's Maximal Exact Matches algorithm (BWA-MEM), producing an arranged and indexed SAM (Sequence Alignment Map) file[139]. This output is transformed using Samtools (https://samtools.github.io) into BAM (Binary Alignment Map) files, the compressed binary version of a SAM file, for better management and efficiency in computation and storage.

Post-alignment processing includes several steps of quality filtering: first, GATK MarkDuplicates is used to identify and remove duplicated reads likely to have originated from duplicates of the original DNA fragments, mitigating potential biases from PCR and library preparation. Lastly, several ML algorithms, such as GATK BaseRecalibrator and GATK ApplyBQSR tools, were applied to set the accuracy of each base call according to the site where it was aligned, and to detect systematic errors made by the sequencing machine[140,141].

Once the BAM files are recalibrated, they will be used as input for variant calling.

3.8.1.1 Variant Calling, Annotation, and Filtering

The GATK HaplotypeCaller tool[139] was used to detect genetic germline variants after performing clean-up operations. The resulting VCF (Variant Call Format) files contained SNV and short InDels (<50bp) from each individual.

The variant quality was assessed by applying hard filtering, followed by the Variant Quality Score Recalibration (VQSR). We filtered the VCFs from each patient using the same parameters as the gnomAD database: Allele balance (AB) ≥ 0.2 and AB ≤ 0.8 (for heterozygous genotypes only), genotype quality (GQ) ≥20, and depth (DP) ≥10 (5 for haploid genotypes on sex chromosomes)[140,141]. After filtering, the VCFs from each patient were combined into a single file using the merge function of BCFtools from Samtools.

This resulted in a VCF file containing multiallelic variants. Then, we split the VCF files into different variants and aligned the indels to the left using the function norm of BCFtools [142].

The merged dataset underwent VQSR, a machine learning tool that calculates a score Variant Quality Score Log-odds (VQSLOD) for each variant, to detect the probability of being a true or false positive. The variants were filtered using a threshold VQSLOD > 90.[143].

Using the Variant Ensembl Variant Predictor v104 (VEP) platform from Ensembl[144], we annotated this file. VEP is a bioinformatics tool that provides information from public databases, such as:

- The consequences of variants, classified as HIGH, MODERATE, MODIFIER, and LOW impact (Table 8).

Table 8. Consequences of variants according to VEP IMPACT categories

VEP IMPACT categories	Variant consequences	
HIGH	• Transcript ablation • Splice acceptor variant • Splice donor variant • Stop gained	• Frameshift variant • Stop lost • Start lost • Transcript amplification
MODERATE	• Inframe insertion • Inframe deletion	• Missense variant • Protein-altering variant
MODIFIER	• Coding sequence variant • Mature miRNA variant • 5 prime UTR variant • 3 prime UTR variant • Noncoding transcript exon variant • Intron variant • NMD transcript variant • Noncoding transcript variant • Upstream gene variant	• Downstream gene variant • TFBS ablation • TFBS amplification • TF binding site variant • Regulatory region ablation • Regulatory region amplification • Feature elongation • Regulatory region variant • Feature truncation • Intergenic variant
LOW	• Splice region variant • Splice donor 5th base variant • Splice donor region variant • Splice polypyrimidine tract variant	• Incomplete terminal codon variant • Start retained variant • Stop retained variant • Synonymous variant

- Variants frequency on different populations, incorporating information from gnomADv3.1 (Genome Aggregation Database)[145].
- Pathogenicity of variants and predictions of deleteriousness, highlighting scores as CADD.
    - ♦ The CADD v1. score is a tool that combines various types of genetic information, such as allelic diversity, conservation, protein functionality, regulatory effects (which have been experimentally tested), pathogenicity, and the severity of associated traits or diseases. The resulting C score is useful in identifying potentially pathogenic diseases, especially when the CADD score is equal to or greater than 20[26].
- Gene conservation and constraint, using scores such as pLI (probability of being Loss of function-intolerant). This score helps to identify the probability that a LoF variant in one allele causes a haplo insufficient phenotype, indicating whether a gene is intolerant (pLI >= 0.9) or tolerant (pLI <= 0.1) to LoF variation[146].

3.8.1.2 Filters Used to Obtain Candidate Genes

Several stringent filters were applied to the total genetic variants identified to identify the candidate genes associated with the familial VM. The following filters were applied sequentially to narrow down the list of candidate genes:

The analysis began by pinpointing genetic variants common to the sequenced family members while absent in any available control subjects. This initial step ensured that the focus was on variants potentially contributing to the disease phenotype rather than those that might be common in the general population.

Then, common variants were filtered out based on their minor allele frequency (MAF) in public databases, including gnomAD global, gnomAD_NFE (Non-Finnish European), and CSVS (Collaborative Spanish Variant Service). Variants with a MAF > 0.1 were excluded. CSVS was only applied to families of Spanish origin. Subsequently, variants were filtered based on their predicted impact. Those classified as "Low" and "Modifier" impact were excluded. Finally, their CADD score, excluding those with a score below 20, further filtered the remaining variants. Additionally, variants found in the Otoscope database, which includes hearing loss genes, were excluded.

3.8.1.3 Expression Basis of Candidate Genes

To investigate the role of the candidate genes in the relevant tissues associated with the VM phenotype, their expression levels were obtained from several datasets.

Firstly, RNA sequencing (RNA-seq) data from human brain tissues were retrieved from the Genotype-Tissue Expression (GTEx) project V8. The brain regions analyzed included the amygdala, anterior cingulate cortex, caudate (basal ganglia), cerebellar

hemisphere, cerebellum, cortex, frontal cortex (BA9), hippocampus, hypothalamus, nucleus accumbens (basal ganglia), putamen (basal ganglia), spinal cord (cervical C-1), and substantia nigra. Gene expression levels were measured in Transcripts Per Million (TPM), with a threshold of 0.5 TPM applied to filter out noise expression.

Secondly, RNA-seq data from adult human inner ear tissues were obtained, including the cochlea, ampulla, saccule, and utricle from patients without hearing loss. This dataset was referenced from a study by Friedman et al [147]. Gene expression levels were reported in Fragments Per Kilobase of exon per Million reads mapped (FPKM), where 1 FPKM corresponds to weak expression, 10 FPKM to moderate expression, and 100 FPKM to high expression [148].

Additionally, RNA-seq data from adult mouse cochlear hair cells and non-hair cells (Pillar and Deiters cells) were obtained from the Gene Expression Analysis Resource (gEAR) portal, specifically the He dataset. The gene expression levels were measured in Reads Per Kilobase of transcript per Million reads (RPKM), and a threshold of 0.5 RPKM was used to avoid noise expression.

Furthermore, RNA-seq data from postnatal day 0 (P0) mouse hair cells and non-hair cells from the cochlea and vestibule were retrieved from the gEAR portal, using the Hertzano dataset. The read counts were normalized by quantiles.

Lastly, microarray data for RNA expression in P0 mouse spiral ganglion neurons (SGN) and vestibular ganglion neurons (VGN) were sourced from the Shared Harvard Inner-Ear Laboratory Database (SHIELD). The expression data were normalized and provided insights into the orthologous genes of the candidate genes being studied. Figure 12 shows a summary of the filters and the order in which they were applied.
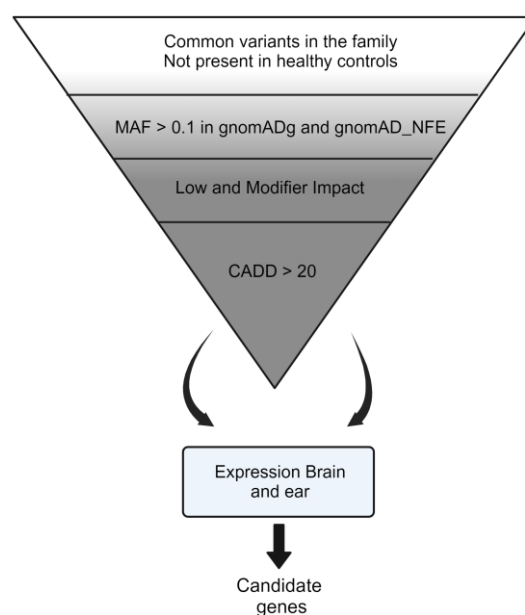
Figure 12. Summary of the filters applied to obtain the candidate variants. *Created with BioRender.com*

3.8.2 Prioritization of Candidate Variants

Different methods were employed to prioritize candidate variants involved in the development of VM. This approach produced a list of the most potentially significant variants for our study.

Initially, we selected genes present in more than one family and genes within the same family that had multiple mutated variants.

After applying the filters described above, we retained variants known to be related to FHM as well as the TRPM7 gene, which has been described in the literature as a candidate gene for VM.

Finally, a manual search of the variants was conducted, focusing on those associated with terms such as "vestibular migraine," "dizziness," "vestibular disorders," "migraine," "pain," or "tinnitus."

3.8.3 Candidate Variant Validation

All candidate variants (SNV and Indels) were validated using the Integrative Genome Viewer (IGV, v2.16.0), a high-performance interactive tool for the visual exploration of genomic data[149].

3.9 Ethical Approval

All patients gave their informed consent for the study (Annex 6-7). The study was carried out according to the principles of the Declaration of Helsinki revised in 2013[150] for investigation with humans and the requirements established in the Spanish legislation in biomedical research, personal data protection, and bioethics. The Granada Ethical Review Board for Clinical Research approved the protocol (Project Acronym: UNITI, Project Number: 848261, H2020-SC1-2019).

4 RESULTS

4.1 Tinnitus Cohort

4.1.1 Description of Both Samples

In this analysis, we present data on 434 patients with tinnitus who were recruited from two sources: 204 patients (46.3%) from the outpatient clinic at the ENT Department and 230 patients (53.7%) from the online sample. The two groups differed significantly in terms of age, sex, and level of education (p-value < 0.001). The average age of the outpatient group was 55 years (range 46-62 years), while the average age of the online group was 44 years (range 37-53 years). Female patients comprised 66% of the online group and 52% of the outpatient group. There were also differences in the clinical features reported by both groups. The frequencies of acoustic trauma, acute otitis media, and presbycusis did not differ significantly between the two groups (p > 0.05). However, sleep disorders (p < 0.001) and metabolic illnesses such as dyslipidemia (p < 0.001) were more common in the outpatient group. Meniere's disease (p < 0.001), vertigo (p < 0.001), and hyperacusis (p < 0.001) were more common in the online group. Anxiety and depression were reported at similar rates in both groups. Table 9 summarizes the demographic characteristics and comorbid conditions of both groups.

Table 9. Demographic data and comorbid conditions for a sample of chronic tinnitus patients (n = 434), including online and outpatient populations

Variable	Online sample (N=230)	Outpatients (N=204)	Corrected P
Age (years)	44 (37 - 53)	55 (46 - 62)	**<0.001**
Sex			**<0.001**
Female	151 (66%)	105 (52.20%)	
Male	79 (34%)	99 (47.80%)	
Body mass Index			>0.050
Underweight	6 (2.60%)	7 (3.70%)	
Normal-weight	110 (47.80%)	79 (41.40%)	
Overweight	72 (31.30%)	70 (36.60%)	
Obesity	42 (18.30%)	35 (18.30%)	
Level of education			**<0.001**
No school	0 (0%)	3 (1.50%)	
Primary school	19 (8.30%)	48 (23.50%)	
Middle school	28 (12.20%)	50 (24.50%)	
High school	44 (19.10%)	41 (20.10%)	
University/higher degree	139 (60.40%)	60 (29.40%)	
Tinnitus family history			>0.050

No	73 (52.10%)	115 (56.40%)	
Three or less relatives	53 (37.90%)	79 (38.70%)	
More than three relatives	14 (10%)	10 (4.90%)	
Ear condition			
Acoustic trauma	12 (5.20%)	21 (10.30%)	>0.050
Acute otitis media	7 (3.10%)	19 (9.30%)	>0.050
Presbycusis	3 (1.30%)	9 (4.40%)	>0.050
SSHL*	22 (9.60%)	16 (7.80%)	>0.050
Meniere's disease	164 (71.60%)	32 (15.70%)	**<0.001**
Hyperacusis	198 (86.1%)	141 (69.10%)	**<0.001**
Vertigo			**<0.001**
Never	30 (13.1%)	62 (30.50%)	
One or less per year	40 (17.50%)	23 (11.30%)	
More than two per year	159 (69.40%)	107 (52.70%)	
No answer	0	11 (5.40%)	
Pain			
Headache	122 (53%)	106 (52%)	>0.050
Neck pain	88 (38.30%)	104 (61.30%)	>0.050
Ear pain	60 (26.10%)	37 (18.10%)	>0.050
Psychiatric conditions			
Anxiety	77 (33.50%)	51 (25%)	>0.050
Depression	39 (17%)	27 (13.20%)	>0.050
Sleep-disorders (start)	49 (21.30%)	74 (36.30%)	**<0.001**
HBP**	22 (9.60%)	34 (16.70%)	>0.050
Dyslipidemia	27 (11.7%)	52 (25.5%)	**<0.001**

*SSHL: sudden sensorineural hearing loss; **HBP: High blood pressure

The study found significant differences in tinnitus duration between the online and outpatient surveys (Table 10). The online survey had a duration of 72 (36-132) months, while the outpatient survey had a duration of 24 (12-96) months. Additionally, the time since tinnitus became bothersome was longer in the online survey, with 48 (24-120) months compared to 24 (12-72) months in the outpatient survey. In terms of the characteristics of perceived tinnitus, the number of sounds reported in the online survey was not statistically significant, but the tinnitus onset was different with a higher tendency to develop a sudden onset online compared to the outpatient survey.

Table 10. Tinnitus characteristics in patients with chronic tinnitus (n = 434)

Variable	Online sample N=230	Outpatients N=204	Corrected P
Tinnitus duration (months)	72 (36 – 132)	24 (12 – 96)	**0.036**
Debilitating tinnitus duration (months)	48 (24- 120)	24 (12 – 96)	>0.050
Worry on tinnitus (%)			>0.050
Severely	93(41.30%)	81 (40.50%)	
Moderately	90 (40%)	76 (38%)	
Slightly	28 (12.40%)	19 (9.50%)	
Not at all	6 (2.70%)	19 (9.50%)	
NA	8 (3.60%)	5 (2.50%)	
Number of sounds			>0.050
One or less	79 (34.60%)	54 (27%)	
More than one	145 (63.60%)	77 (38.50%)	
No answer	4 (1.70%)	69 (34.50%)	
Type of onset			**<0.001**
Sudden	84 (36.50%)	113 (56.50%)	
Gradual	87 (37.20%)	78 (39%)	
No answer	55 (24.30%)	9 (4.40%)	
Triggers			
Changes in hearing	43 (19.40%)	19 (9.50%)	>0.050
Ear fullness	83 (37.40%)	50 (25.10%)	>0.050
Stress	59 (26.60%)	46 (23.10%)	>0.050
Tinnitus increasing factors	208 (92.40%)	135 (69%)	0.054
Tinnitus reducing factors	159 (71.30%)	112 (57.40%)	**<0.001**
Treatment (Yes/No//NA)	41.4/58.6 (%)	21.1/75.9 //3 (%)	**<0.001**

Regarding factors that influence tinnitus, the number of individuals reporting aggravating and mitigating factors on tinnitus was higher in the online group compared to the outpatient survey. However, these differences were only statistically significant for the higher mitigating factors in the online group. Tinnitus-increasing factors include situations or conditions that could worsen tinnitus, such as lack of sleep, stress, or alcohol/coffee consumption. Conversely, tinnitus-reducing factors could decrease the intensity of tinnitus or improve its perception.

The study also analyzed the number of treatments used for tinnitus. The results showed that more treatments were used in the online survey. The types of treatments used included sound therapy, cognitive behavioral therapy, and medication. However, there were no differences between all the treatment subgroups, indicating that the types of treatments used are consistent across both online and outpatient populations.

4.1.2 Age and Sex-Adjusted Comparison of Both Surveys

In the second part of the analysis, we adjusted both samples by age and sex to compare clinical and psychoacoustic variables. This resulted in a sample size of 344 individuals, consisting of 204 outpatients and 140 respondents from an online survey. The average age of the online group was 52 (ranging from 46 to 57.75), and the outpatient cohort had an average age of 55 (ranging from 46 to 62). The online group predominantly comprised women (62%), while women represented 52.2% of the outpatient cohort.

Furthermore, we found that the level of education was significantly higher in online respondents compared to outpatients (p < 0.001). There were differences between outpatients and online participants in terms of clinical profile. MD (75%, p < 0.001), vertigo (p < 0.001), and hyperacusis (88%, p < 0.001) were more commonly reported in the online survey. However, hyperacusis was also frequently reported in outpatients (69%). Table 11 compares both groups' demographic data and comorbid conditions after adjusting for sex and age.

Table 11. Demographic data and comorbid conditions in patients with chronic tinnitus after adjusting for sex and age (n = 344)

Variable	Online sample (N=140)	Outpatients (N=204)	Corrected P
Age (years)	52 (46 – 57.75)	55 (46 – 62)	>0.050
Sex			>0.050
Female	86 (62%)	105 (52.20)	
Male	53 (38%)	96 (47.80%)	
Body mass Index			>0.050
Underweight	3 (2.10%)	4 (2.10%)	
Normal-weight	62 (44.30%)	82 (43%)	
Overweight	45 (32.10%)	70 (36.60%)	
Obesity	30 (21,40%)	35 (18,30%)	
Level of education			**<0.001**
No school	0 (0%)	3 (1.50%)	
Primary school	16 (11.40%)	48 (23.50%)	
Middle school	20 (14.30%)	50 (24.50%)	
High school	31 (22.10%)	41 (20.10%)	
University/higher degree	73 (52.10%)	60 (29.40%)	
Tinnitus family history			>0.050
No	73 (52.10%)	115 (56.40%)	
Three or less relatives	53 (37.90%)	79 (38.70%)	
More than three relatives	14 (10%)	10 (4.90%)	
Ear condition			
Acoustic trauma	8 (5.70%)	21 (10.30%)	>0.050
Acute otitis media	5 (3.60%)	19 (9.30%)	>0.050

Presbycusis	3 (2.10%)	9 (4.40%)	>0.050
SSHL*	14 (10%)	16 (7.80%)	>0.050
Meniere disease	104 (75%)	32 (15.70%)	**<0.001**
Hyperacusis	198 (88%)	141(69.10%)	<0.001
Vertigo			**<0.001**
Never	17 (12.1%)	62 (30.5%)	
One or less per year	22 (15.7%)	23 (11.3%)	
More than two per year	101 (72.1%)	107 (52.7%)	
No answer	0	11 (5.4%)	
Pain			
Headache	68 (48.6%)	106 (52%)	>0.050
Neck pain	49 (35%)	104 (51%)	0.054
Ear pain	35 (25%)	37 (18.10%)	>0.050
Psychiatric conditions			
Anxiety	44 (31.4%)	51 (25%)	>0.050
Depression	21 (15%)	27 (13.20%)	>0.050
Sleep-disorders Start	32 (23%)	74 (36.50%)	>0.050
HBP**	20 (14.30%)	34 (16.70%)	>0.050
Dyslipidemia	22 (15.7%)	52 (25.50%)	0.054

*SSHL: sudden sensorineural hearing loss; **HBP: High blood pressure

There were differences in the time since the onset of tinnitus between the online survey group and the outpatient group. The online survey group had experienced tinnitus for a longer period (96 months on average) compared to the outpatient group (24 months on average), which was statistically significant ($p < 0.001$; Table 12). The time until the development of disabling tinnitus was also longer for the online group (60 months on average) compared to the outpatient group (24 months on average), which was also statistically significant ($p = 0.004$). Moreover, there were differences in the number of perceived sounds in both groups. Outpatients usually reported experiencing one sound, while patients from the online survey usually reported experiencing two or more sounds, which was statistically significant ($p < 0.001$). No significant difference was found between both groups regarding triggers associated with tinnitus, except for change in hearing, which was more frequently reported in the online survey ($p = 0.002$). Aggravating factors were also more reported by the online group than by outpatients ($p < 0.001$).

Some variables were statistically significant in the whole sample but were not significant after age and sex adjustment. These variables included sleep disorders and dyslipidemia between the comorbid conditions. However, the tinnitus characteristics that were significantly different between both samples were the type of onset, mitigating factors, and treatment necessity. When both groups were adjusted for age and sex, the

differences were the duration of disturbing tinnitus, the number of sounds, changes in hearing, and aggravating factors.

The study used K-means clustering to divide the sample into two groups: one included 160 individuals aged between 19 and 53 (the "young" group; 80 online participants and 79 outpatients), and the other included 152 individuals aged between 54 and 94 (the "old" group; 52 online participants and 98 outpatients). The clusters were balanced in size and did not have any outliers. The statistical comparisons between the online and outpatient surveys were performed using the chi-squared test for each variable and cluster. The significance p-values were adjusted for multiple comparisons using the Bonferroni correction, yielding adjusted p-values of 0.0053, 0.00263, and 0.0005 for significance levels of 0.1, 0.05, and 0.01, respectively.

Table 12. Tinnitus characteristics in hospital outpatients and online survey participants after adjusting for sex and age (n = 344)

Variable	Online sample (N=140)	Outpatients (N=204)	Corrected P
Tinnitus duration (months)	96 (36 - 180)	24 (12 - 93)	**<0.001**
Debilitating tinnitus (months)	60 (24 - 129)	24 (12 – 60)	**0.036**
Worry on tinnitus			>0.050
Severe	58 (41.70%)	81 (40.50%)	
Moderate	52 (37.40%)	76 (38%)	
Slight	19 (13.70%)	19 (9.50%)	
Not at all	4 (2.90%)	19 (9.50%)	
No answer	6 (4.30%)	5 (2.50%)	
Number of sounds			**<0.001**
One or less	47 (34%)	113 (56.50%)	
More than one	89 (64%)	72 (39%)	
No answer	3 (2%)	9 (4.50%)	
Type onset			>0.050
Sudden	49 (35.80%)	54 (27%)	
Gradual	58 (42.30%)	77 (38.50%)	
No answer	30 (21.90%)	69 (34.50%)	
Triggers			
Changes in hearing	31 (23.10%)	19 (9.50%)	**0.018**
Ear fullness	40 (30%)	50 (25.10%)	>0.050
Stress	30 (22.40%)	46 (23.10%)	>0.050
Tinnitus increasing factors	128 (92.80%)	135 (69%)	**<0.001**
Tinnitus reducing factors	92 (67.60%)	112 (57.40%)	>0.050
Treatment (Yes/No//NA)	36.8/63.2 (%)	21.1/75.9//3 (%)	0.054

The results showed that the frequency of ear problems reported in both the young and old clusters significantly differed between the online and outpatient surveys at the adjusted significance level of 0.00263. The occurrence of MD was also significantly different between the online and outpatient cohorts in both clusters of young and old patients (p = 5.18 × 10^{-12} for the young and p = 1.39 × 10^{-13} for the old cluster). Nonetheless, hyperacusis showed a significant difference only between online and outpatient cohorts in the cluster of young patients. Moreover, the online group reported higher rates of hyperacusis in this condition. However, after analyzing the intensity of hyperacusis, it was found that older respondents were more likely to report severe hyperacusis in the online survey (p = 0.001) (Table 13).

Table 13. Comparison of the main clinical variables in the ESIT-SQ according to the age of patients with chronic tinnitus.

Variable	Cluster	χ2	OR (95% CI)	Corrected P
Sex	Young	3.323	0.53 (0.28-1.00)	0.680
	Old	0.128	0.83 (0.42-1.64)	0.721
Education Level	Young	7.961	0.39 (0.20-0.77)	0.047
	Old	10.448	0.37 (0.18-0.76)	0.015
Otological disease	Young	26.166	**0.05 (0.01-0.20)**	**<0.001**
	Old	23.828	**0.05 (0.01-0.22)**	**<0.001**
Acoustic trauma	Young	5.472	6.31 (1.35-29.47)	0.019
	Old	0.001	1.19 (0.35-4.06)	0.975
Presbyacusis	Young	0.000	0.50 (0.04-5.62)	0.991
	Old	0.900	3.85 (0.46-32.16)	0.343
Acute Otitis	Young	1.617	2.89 (0.74-11.33)	0.203
	Old	0.698	2.48 (0.51-11.92)	0.404
Neck Pain	Young	0.004	1.08 (0.57-2.04)	0.950
	Old	7.251	2.74 (1.36-5.51)	0.007
Sleep Disorder	Young	1.325	1.64 (0.79-3.41)	0.250
	Old	1.328	1.62 (0.79-3.30)	0.249
High blood pressure	Young	0.049	0.79 (0.29-2.11)	0.824
	Old	0.011	1.15 (0.49-2.66)	0.917
Low blood pressure	Young	0.000	1.37 (0.30-6.32)	0.986
	Old	0.000	1.21 (0.36-4.14)	0.997
Cholesterol	Young	0.269	1.46 (0.55-3.83)	0.604
	Old	1.645	1.21 (0.35-4.14)	0.200
Meniere's Disease	**Young**	47.614	**0.08 (0.03-0.17)**	**<0.001**
	Old	54.719	**0.05 (0.02-0.12)**	**<0.001**
Hyperacusis (yes/no)	**Young**	9.198	**0.27 (0.11-0.61)**	**0.002**
	Old	4.703	0.34 (0.14-0.85)	0.030
Severity of hyperacusis	Young	0.029	1.17 (0.52-2.65)	0.864
	Old	11.518	**4.06 (1.84-8.97)**	**0.001**

Anxiety	Young	0.002	0.96 (0.48-1.91)	0.960
	Old	3.231	0.47 (0.22-0.99)	0.072
Depression	Young	0.756	0.61 (0.24-1.49)	0.385
	Old	0.051	1.25 (0.48-3.27)	0.822
Antidepressant	Young	0.000	1.00 (0.24-4.15)	1.000
	Old	0.204	1.93 (0.39-9.66)	0.651
Familial history of tinnitus	Young	1.088	0.68 (0.36-1.27)	0.297
	Old	0.017	0.99 (0.50-1.94)	0.896

4.1.3 Stratified Analysis for Meniere's Disease

We combined all the online and outpatient surveys and categorized them based on the presence of MD. We compared each variable using t-tests or χ2-tests as appropriate. These analyses revealed statistically significant differences between both groups with respect to age, sex, and history of acute otitis media, vertigo, and hyperacusis (Table 14).

Table 14. Demographic data and comorbid conditions were analyzed in 344 patients with chronic tinnitus after stratifying for Meniere's disease

Variable	Meniere's disease (N=136)	Non-MD (N=208)	Corrected P
Age (years)	52.26 ± 8.62	53.23± 12.75	**<0.001**
Sex			**<0.001**
Female	85 (62.5%)	105 (50.70%)	
Male	51 (37.5%)	98 (47.30%)	
Body mass Index			>0.050
Underweight	1 (0.70%)	7 (2.10%)	
Normal-weight	62 (45.60%)	81 (41.80%)	
Overweight	43 (31.60%)	72 (37.1%)	
Obesity	30 (22.10%)	35 (18%)	
Level of education			>0.050
No school	0 (0%)	3 (1%)	
Primary school	23 (16.90%)	64 (18.70%)	
Middle school	21 (15.40%)	70 (20.40%)	
High school	24 (17.60%)	72 (21%)	
University/higher degree	68 (50%)	132 (38.50%)	
Tinnitus family history			>0.050
No	74 (54.40%)	188 (54.80%)	
Three or less relatives	52 (38.20%)	132 (38.50%)	
More than three relatives	10 (7.4%)	23 (6.70%)	
Ear condition			
Acoustic trauma	5 (3.70%)	24 (11.60%)	>0.050

Acute otitis media	2 (1.50%)	22 (10.60%)	**0.018**
Presbycusis	4 (3%)	8 (4%)	>0.050
SSHL*	11 (8.10%)	19 (9.20%)	>0.050
Hyperacusis	122 (90%)	141 (68.10%)	**<0.001**
Vertigo			**<0.001**
Never	2 (1.5%)	77 (37.2%)	
One or less per year	16 (11.9%)	29 (14%)	
More than two per year	116 (85.9%)	91 (44%)	
No answer	1 (0.7%)	10 (4.8%)	
Pain			
Headache	75 (55.1%)	98 (47.3%)	>0.050
Neck pain	50 (36.80%)	103 (50%)	>0.050
Ear pain	31 (22.80%)	41 (20%)	>0.050
Psychiatric conditions			
Anxiety	39 (28.70%)	56 (27.10%)	>0.050
Depression	22 (16.2%)	26 (12.60%)	>0.050
Sleep-disorders start	33 (24.30%)	72 (35%)	>0.050
HBP**	18 (13.20%)	36 (17.40%)	>0.050
Dyslipidemia	23 (17%)	51 (25%)	>0.050

*SSHL: sudden sensorineural hearing loss; **HBP: High blood pressure

The tinnitus profile varied significantly in individuals with MD as compared to those without it (Table X). Patients with MD had higher tinnitus duration, duration of debilitating tinnitus, worry about tinnitus, and number of sounds perceived. It is worth noting that changes in hearing (OR = 3.39, p < 0.001) and the description of factors that increase tinnitus (OR = 3.95, p < 0.001) were the most frequently reported symptoms in MD patients.

Table 15. Tinnitus features in patients with chronic tinnitus after stratifying for Meniere's disease (n = 344)

Variable	Meniere's disease (N=136)	Non-MD (N=208)	Corrected P
Tinnitus duration (months)	125.84 ± 108	55.29 ± 87.47	**<0.001**
Debilitating tinnitus duration (months)	106.55 ± 99.60	44.04 ± 78.25	**<0.001**
Worry on tinnitus			0.018
Severely	69 (50.70%)	70 (34.70%)	
Moderately	49 (36%)	78 (38.6%)	
Slightly	12 (8.80%)	26 (12.90%)	
Not at all	1 (0.70%)	22 (10.90%)	
No answer	5 (3.70%)	6 (3%)	
Number of sounds			**<0.001**
One or less	44 (32.40%)	115 (56.9%)	

More than one	89 (65.40%)	78 (38.60%)	
No answer	3 (2.20%)	9 (4.50%)	
Type of onset			>0.050
Sudden	43 (31.60%)	60 (30%)	
Gradual	64 (47.10%)	70 (35%)	
No answer	29 (21.30%)	70 (35%)	
Triggers			
Changes in hearing	32 (24.10%)	17 (8.50%)	**<0.001**
Ear fullness	37 (27.80%)	52 (26.10%)	>0.050
Stress	30 (22.60%)	45 (22.60%)	>0.050
Tinnitus increasing factors	123 (90.40%)	139 (70.6%)	**<0.001**
Tinnitus reducing factors	89 (66%)	114 (59%)	>0.050
Use of any treatment for tinnitus	52 (39%)	42 (20%)	**<0.001**

4.2 Vestibular Migraine Cohort

4.2.1 Patient demographics

Fifty-one patients were initially selected for the study, but only 50 were included due to incomplete information from one participant. Table 16 summarizes the demographic characteristics and comorbid conditions of our patient group. Out of the 50 patients, 35 (70%) were diagnosed with definite VM, while the remaining 15 (30%) were diagnosed with probable VM. Our study group comprised of 45 females and 5 males, with an average age of 46.40 ± 13.74 years (range 18-71 years). On average, the patients had been experiencing VM for 8+/- 8.52 years. During the disease, most patients reported headaches (96%), neck pain (68%), insomnia (40%), or temporomandibular joint dysfunction (40%).

Patients with VM and tinnitus were more likely to experience insomnia. When comparing the characteristics of VM patients with and without tinnitus, a statistically significant difference was observed in the frequency of sleep disorders (OR= 4.65 (CI 1.23-17.67); X2= 5.21, p=0.02) and fall asleep disorder (OR= 6.88 (CI 1.35-35.11); X^2= 6.35, p=0.012). No significant differences were found for the other variables.

Table 16. Demographic variables in patients with VM

Variable	VM	Variable	VM
Diagnostic state (%)		**Pain**	
Defined VM	35 (70%)	Headache	48 (96%)
Probable VM	15 (30%)	Neck pain	34 (68%)
		Ear pain	14 (28%)
		TMJ	20 (40%)
		Facial pain	5 (10%)
Age (mean ± SD)	46.40 ± 13.74	**Oral problems**	
Onset VM (mean ± SD)	39 ± 13.10	TMJ disorders	19 (38%)
VM duration (mean ± SD)	8 ± 8.52	Dental problems	13 (16%)
Sex (% Female)	45 (90%)		
Body mass Index (%)		**Psychiatric conditions**	
Underweight	1 (2%)	Anxiety	21 (42%)
Normal weight	23 (46%)	Depression	11 (22%)
Overweight	16 (32%)	Excessive stress	11 (22%)
Obesity	10 (20%)		
Level of education (%)		**Sleep-disorders**	
No school	1 (2%)	Falling asleep	20 (40%)
Elementary	4 (8%)	Staying asleep	8 (18%)
Middle school	9 (18%)		
High school	11 (22%)		
University	25 (50%)		
Alcohol		**Cardiovascular disorders**	
No alcohol	34 (68%)	HBP	16 (32%)
≤ 2 drinks/day	11 (22%)	LBP	5 (10%)
> 2 drinks/day	5 (10%)		
Smoking status		**Metabolic disorders**	
Never	31 (62%)	Thyroid disorder	12 (24%)
Current Smoker	8 (16%)	Diabetes	3 (6%)
Ex-smoker	11 (22%)	Increased Cholesterol	16 (32%)
Otological disease			
Acoustic trauma	1 (2%)		
Acute otitis media	7 (14%)		
Ear surgery	1 (2%)		
Dental surgery	15 (30%)		
Neurosurgery	2 (4%)		
Hyperacusis	30 (60%)		

TMJ - Temporomandibular joint; HBP - high blood pressure; LBP - low blood pressure

4.2.2 Patients with VM and tinnitus

Out of the 50 patients who participated in the study, 38 of them (76%) suffered from tinnitus of some form. It should be noted that tinnitus was permanent for around 34% of the patients with VM and intermittent for the rest of the individuals. Only one patient (2%)

had pulsatile tinnitus and was excluded from the rest of the psychometric assessments (Figure 13A).

The clinical and psychoacoustic features of tinnitus were obtained in 35 out of 37 VM patients who had tinnitus (Table 17). Two patients who did not complete the assessment were also excluded. On average, the time since the onset of tinnitus was 8.80 ± 9.38 years (105.70 ± 112.50 months), while the mean time of tinnitus that was considered annoying was 6.30 ± 7.28 years (75.57 ± 87.31 months).

Table 17. Clinical and psychoacoustic features of patients with VM and tinnitus

Variable	VMt N=35
Onset tinnitus (months; mean ± SD)	105.70 ± 112.50
Disabling tinnitus duration (months; mean ± SD)	75.57 ± 87.31
Worry on tinnitus (%)	
Moderately	8 (24.2%)
Slightly	7 (21.2%)
Not at all	9 (27.3%)
Do not know	1 (3%)
Number of sounds (%)	
One or less	16 (48.5%)
More than one	16 (48.5%)
Do not know	1 (3%)
Type of onset (%)	
Sudden	10 (31.3%)
Gradual	11 (34.4%)
Do not know	11 (34.4%)
Tinnitus sound-like (%)	
Tonal	11 (34.4%)
Noise-like	10 (31.3%)
Music-like	1 (3.1%)
Crickets	4 (12.5%)
Others	6 (18.8%)
Pitch of tinnitus (%)	
High pitched	14 (42.4%)
Medium pitched	9 (27.3%)
Low pitched	10 (30.3%)
Tinnitus location (%)	
Right ear	5 (15%)
Left ear	5 (15%)
Both	21 (64%)
Do not know	2 (6%)
Triggers (%)	20 (57.1%)*
Fullness	11 (33%)
Stress	7 (21%)

Neck trauma	4 (12%)
Infection	8 (24.2%)
Pressure changes	8 (24.2%)
Noise exposure	5 (15.2%)
Tinnitus increasing factors (%)	19 (58%)
Tinnitus reducing factors (%)	18 (56%)
Treatment (%)	4 (12%)

* Shows the prevalence of patients with any trigger; VMt: Vestibular Migraine and Tinnitus

4.2.3 Psychometric Assessment

Out of 37 patients with VM and tinnitus, 35 completed the THI test. The mean THI score was 30.29 ± 29.43, with a wide range of scores from 0 to 92. Although the median score was 16, it is worth noting that six patients (17%) had THI scores >58, which is considered severe or catastrophic (as shown in Figure 13B).

60% of the patients reported experiencing hyperacusis. Of the 50 patients who completed the HSQ, 26% (13/50) reported severe or very severe disability (Figure 13C). The mean HSQ score was 11.86 ± 11.27, with a minimum score of 0 and a maximum of 40.

The overall HADS score was 13.28 ± 7.34. More specifically, 53% of patients displayed clinical anxiety symptoms (HADSa>8), while 25% of patients presented depressive symptoms (HADSd>8).
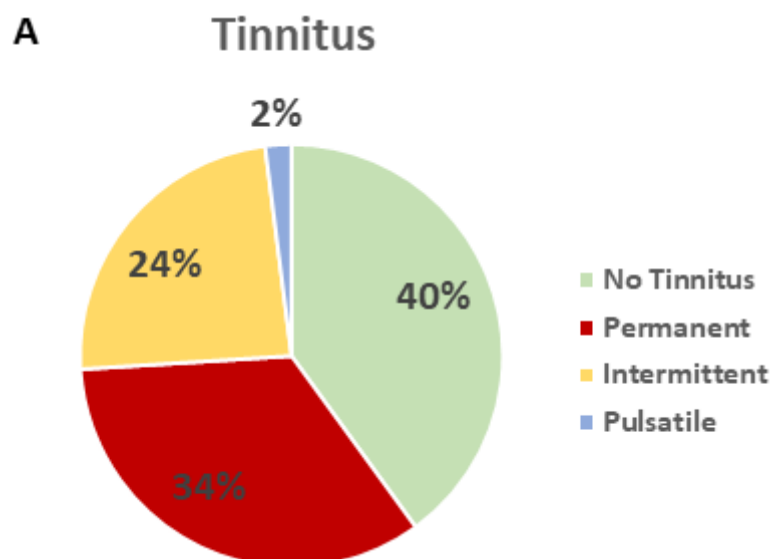
Figure 13. Frequency of permanent, intermittent, and pulsatile tinnitus in VM patients (A).

B THI

- Slight problem (0-16)
- Mild (18-36)
- Moderate (38-56)
- Severe (58-76)
- Catastrophic (>78)

2,80%
14,30%
17,20%
14,30%
51,40%

C HSQ-GÜF

- Slight sensitivity (0-10)
- Moderate sensitivity (11-17)
- Severe sensitivity (18-25)
- Vere severe sensitivity (26-45)

16%
10%
14%
60%
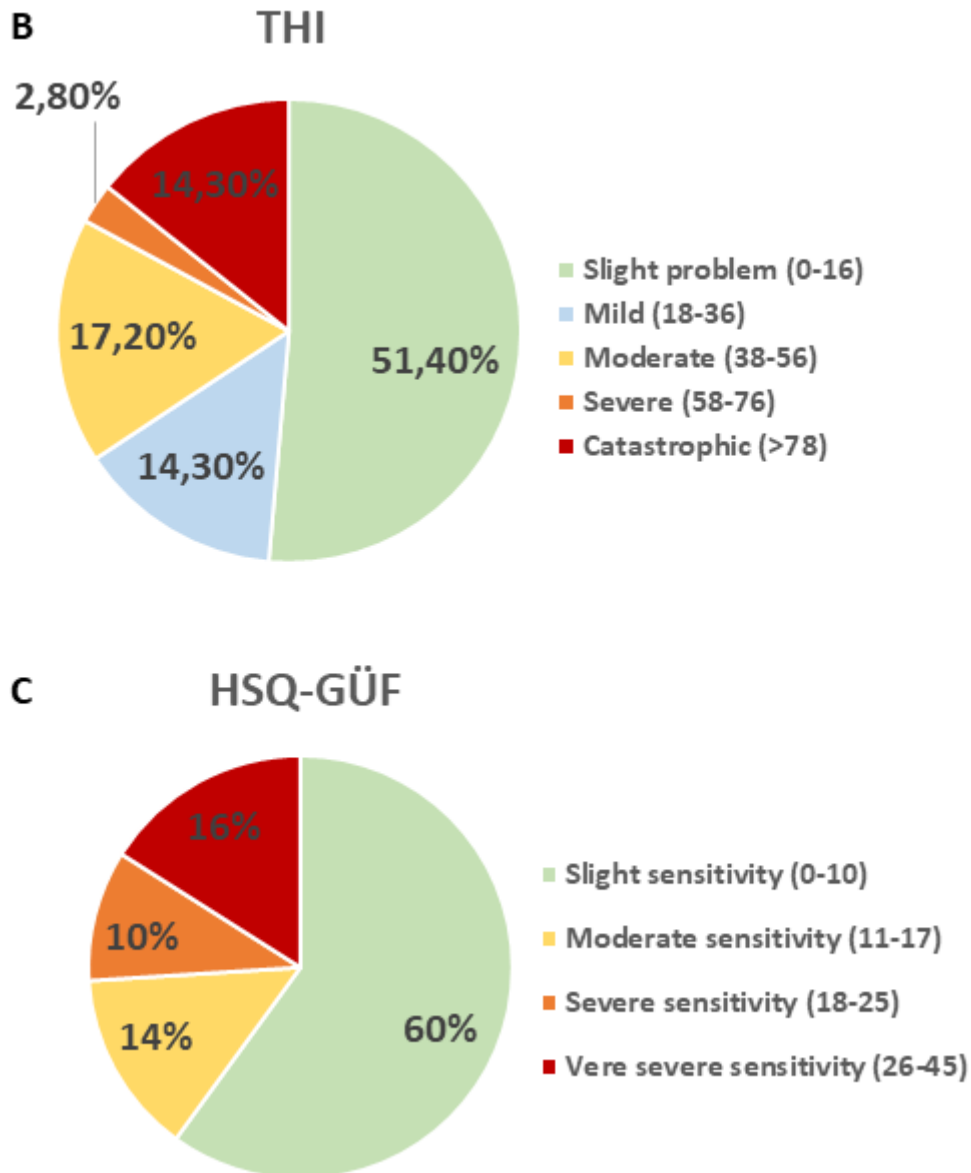
Figure 13 (continued). THI score to assess the functional impact of tinnitus (B) and GUF score for hyperacusis (C).

4.2.4 Hearing Status

The pure tone average (PTA) hearing threshold (500-4000Hz) was found to be 15 ± 11 dB and 15 ± 12 dB in the right and left ears, respectively. The PTA threshold was not significantly different in patients with tinnitus (16 ± 16 dB in the right ear and 17 ± 14 dB in the left ear).

Additionally, when we compared hearing thresholds in both ears, we found that 8% of patients had hearing loss (> 25dB) in the right ear and 10% in the left ear. When we stratified VM patients based on whether they had tinnitus or not (VMt vs VMo), we observed that only 12% of patients with tinnitus had hearing loss.

Figures 14 and 15 show a box plot that displays the hearing thresholds for both right and left ears. Most patients were not able to perceive higher frequencies within the safety range, so this data is missing. The frequencies used to calculate the mean for each frequency are shown in Table 18.
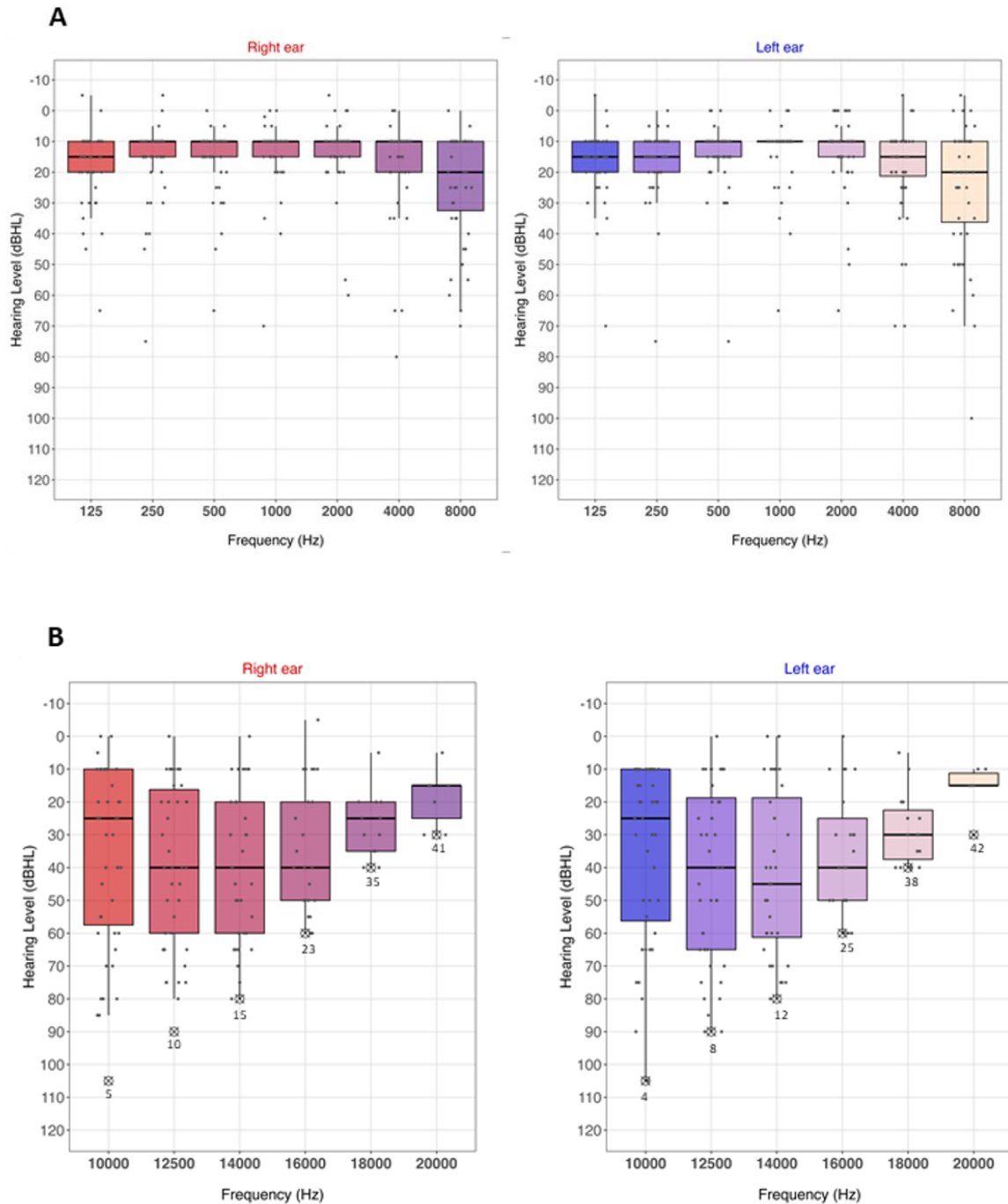
Figure 14. Box plot representing hearing thresholds in the VM individuals. Standard audiogram (125-8000Hz) (A) and high frequencies (8000-20000Hz) (B). ⊗ Individuals unable to respond to high frequencies.
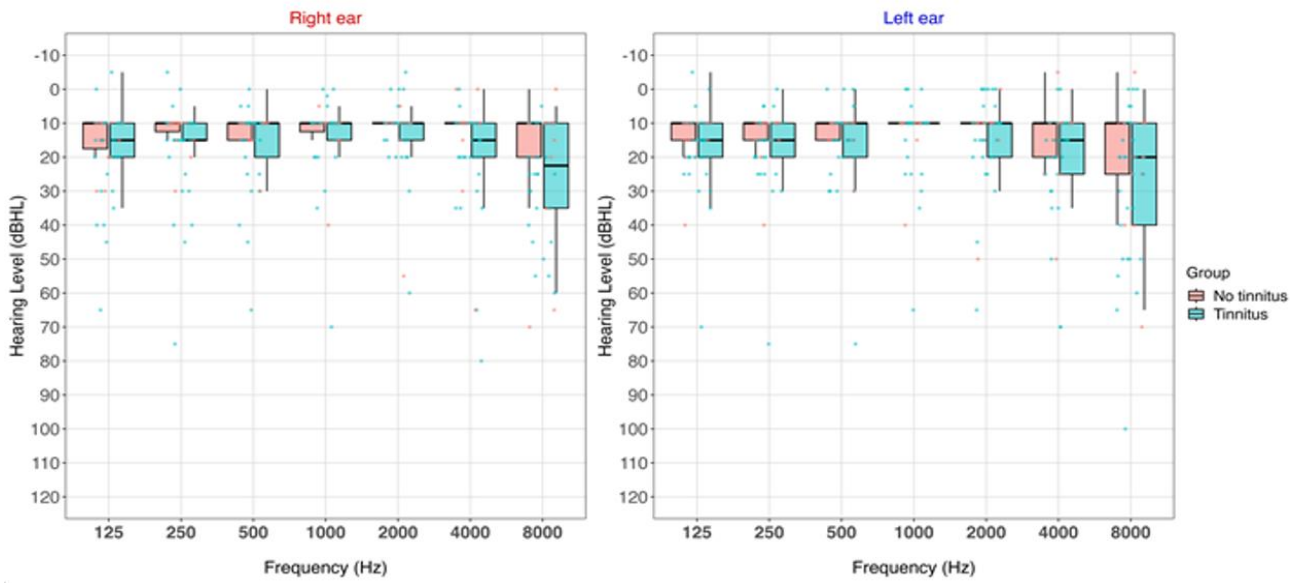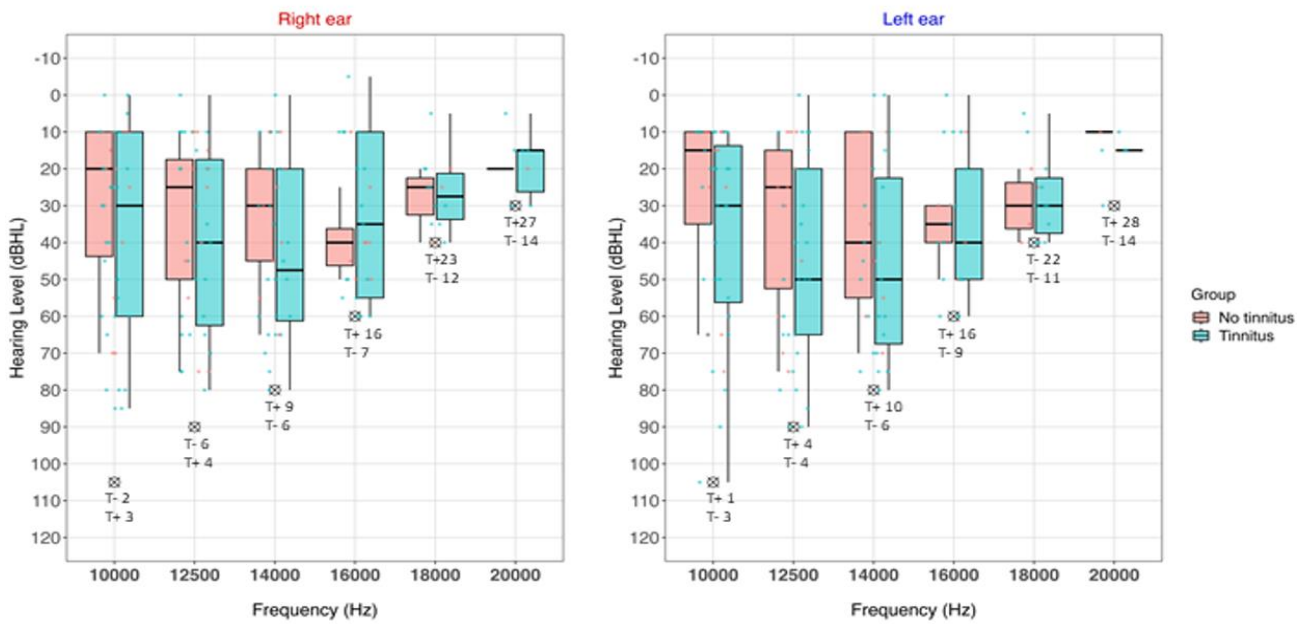
Figure 15. Box plot representing hearing loss in the VM with and without tinnitus in the standard (125-8000Hz) (A) high frequencies audiograms (8000-20000Hz) (B). The number of patients with (T+) or without tinnitus (T-) who had no response within the permitted hearing range is indicated. ⊗ Individuals unable to respond to high frequencies.

Table 18. Responses to pure tones in individuals with VM, according to the frequency, ear, and presence or absence of tinnitus.

Frequency	Right ear (n patients)			Left ear (n patients)		
	Total	Tinnitus	No-tinnitus	Total	Tinnitus	No-tinnitus
125 Hz	48	33	15	48	33	15
250 Hz	48	33	15	48	33	15
500 Hz	48	33	15	48	33	15
1000 Hz	48	33	15	48	33	15
2000 Hz	48	33	15	48	33	15
4000 Hz	48	33	15	48	33	15
8000 Hz	47	32	15	48	33	15
10000 Hz	43	31	12	44	32	12
12500 Hz	38	27	11	40	29	11
14000 Hz	33	24	9	36	23	9
16000 Hz	25	17	8	23	17	6
18000 Hz	13	10	3	15	11	4
20000 Hz	7	6	1	6	5	1

Seventeen patients who experienced audible tinnitus were selected to determine if they showed any signs of residual inhibition. The study found that tinnitus disappeared for over a minute in six patients and for less than a minute in two patients. Tinnitus intensity decreased in two subjects; six reported no change, and one patient described worse tinnitus intensity. The study also found that 8000Hz was the most common frequency (pitch) for tinnitus, while the right ear was the most common location of tinnitus (23.5%) over bilateralism (17.6%). However, most patients were unable to locate the source of their tinnitus.

4.2.5 Correlation matrix and linear regression

Several noteworthy associations were observed among the variables of the psychometric tests, as presented in Table 19. A statistically significant positive correlation (ρ= 0.57) was found between THI and HSQ-GÜF. Additionally, positive correlations were observed between the THI score against HADSa and HADSd with values of ρ=0.441 and ρ=0.552, respectively.

Table 19. Spearman's correlations between the questionnaires' scores

Variable	THI score	HSQ score	HADS-a
HSQ score	0.574**		
HADS-a score	0.441**	0.521**	
HADS-d score	0.552**	0.40**	0.662**

** p <0.01

Similarly, both subgroups showed a statistically significant positive correlation between the HSQ-GÜF and the HADS scale, with ρ=0.522 for HADSa and ρ=0.40 for HADSd. Additionally, a positive correlation (ρ=0.662) was observed between HADSa and HADSd (all p-values ≤ 0.01). The linear regression plot in figure 16 displayed that higher scores on THI or HSQ-GÜF were significantly associated with higher scores on both the HADSa and HADSd scales.
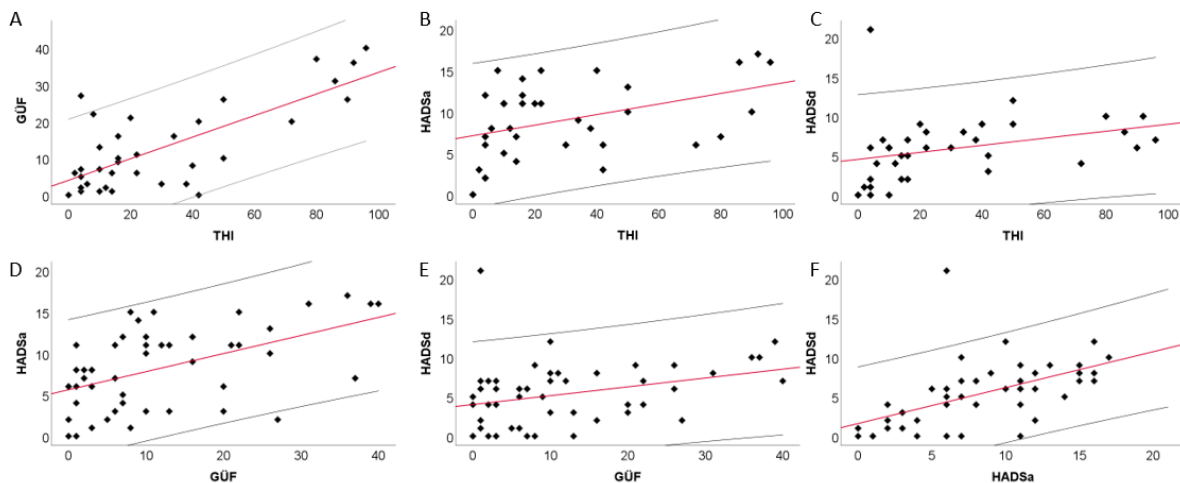
Figure 16. Scatter plots showing the relationship between THI and GÜF (A), THI and HADSa (B), THI and HADSd (C), GÜF and HADSa (D), GÜF and HADSd (E) and HDASa and HADSd (F).

4.2.6 Contributing factors associated with tinnitus in VM patients

Multiple linear regression showed a statistically significant relationship between THI, HSQ-GÜF, and HADSa scores. The regression model explains a substantial proportion of variance (R^2 = 0.56, $F_{(2, 32)}$ = 20.39, p < 0.001, adj. R^2 = 0.53). Both variables were positively related, but only HSQ-GÜF was significant (p=0.001).

4.3 VM familial cohort

4.3.1 Clinical evaluation of familiar VM and affected relatives or controls

Table 20 summarizes the clinical characteristics of the families. The clinical symptoms were very variable between individuals of different families and individuals within the same family. Some individuals experienced migraines strongly related to menstruation (Fam 1 I-1 and 1 II-1, Fam 2 II-1 and II-2, and Fam 4 II-1). The aura was present in several individuals; all of them described it as a visual aura. An individual had a nystagmus crisis during a migraine attack (Fam 3 II-1).

Vestibular symptoms exhibited significant duration variability among individuals, as well as the nature of the dizziness experienced. Patients reported a spectrum of symptoms,

ranging from rotatory vertigo to imbalance induced by body movements. Additionally, neurovegetative symptoms were frequently observed in several individuals during episodes of dizziness.

Tinnitus was a common symptom in these patients. Intermittent tinnitus was observed in Family 1 II-1, Family 2 II-1 and II-2, and Family 4 III-1 and III-2, while permanent tinnitus was reported in Family 1 I-1 and II-2. Self-reported hyperacusis was present in half of the patients who completed the questionnaires. Only one patient reported hearing loss in the audiogram (Fam 1 I-1), while another individual, Fam 2 II-1, indicated hypoacusis during the anamnesis.

The tinnitus reported by all patients had a THI score of less than 10, indicating a slight handicap or none. Hyperacusis, when present, did not exceed a GÜF score of 17. The Khalfa Hyperacusis Questionnaire, which was completed by the Italian population, had an average score of 14.8 ± 6.60. The mean of HADSa was 7.81 ± 7.77, and the mean of HADSd was 4.80 ± 2.94.

4.3.2   Description and characterization of each family

4.3.2.1   Family 1

Four individuals were studied: two have definite VM (II-1 and II-2), one has VM (I-1), and the other has only developed migraine (III-3).

- Patient I-1 is a 65-year-old woman with probable VM. She is the mother of the two subjects with the complete phenotype (II-1 and II-2). Symptoms began at the age of 45. Notably, the migraine is characterized by the absence of aura. The migraine has progressively decreased in intensity with menopause. Vestibular symptoms include episodes of spinning sensation and movement-induced instability, accompanied by tinnitus and SNHL at frequencies greater than 8000Hz. She has permanent tinnitus without impacting on her quality of life. Questionnaires scores: THI 0, GÜF 0, HADSa 0 and HADSd 0.

- Subject II-1 is a 43-year-old woman with a complete VM phenotype. She reports the onset of symptoms at the age of 16. Her migraine is characterized by hemicranial pain, with aura lasting hours, accompanied by phonophobia and photophobia. She notes a correlation between migraine symptoms and menstruation. Vestibular symptoms include instability, vomiting, and movement-induced dizziness. Unilateral intermittent tinnitus in right ear, not other auditory symptoms. Questionnaires scores: THI 4, GÜF 7, HADSa 18 and HADSd 6.

- Subject II-2 is a 38-year-old woman who experienced onset of symptoms at the age of 35. She reports migraines with aura lasting up to 24-48 hours. Vestibular symptoms

are described as either a sensation of objects spinning for hours or feelings of unsteadiness and dizziness induced by head motion. She reports permanent bilateral tinnitus with low impact in her life and hyperacusis. There are no reports of SNHL or other audiological symptoms. Questionnaires scores: THI 6, GÜF 3 HADSa 8 and HADSd 4

- Subject III-1 is an 8-year-old male diagnosed with migraine without vestibular symptoms. There are no reported audiological symptoms.

All individuals were asymptomatic during the medical interview.

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4.3.2.2 Family 2

The second family included eight sequenced individuals, though not all completed the questionnaires. For some individuals, only clinical information could be collected during the anamnesis.

- The patient, II-1, is the older sister of the proband. She is a 62-year-old woman who reports the onset of migraines at age 25 and dizziness at age 40, describing it as instability that worsens with movement. For the past 20 years, she has experienced both symptoms together. She also has age-related hearing loss, intermittent tinnitus and no other associated symptoms. Questionnaires scores: THI: 10, GÜF 13, HADSa 11 and HADSd 0.

- The patient, II-2, is a 54-year-old woman with a history of migraines since her youth (20 years old). The frequency of migraine attacks has decreased with menopause. She does not report symptoms of dizziness or imbalance; she does not present tinnitus or hearing loss. No other otologic symptoms are described. Questionnaires scores: GÜF 1, HADSa: 15 and HADSd 8.

- The proband, II-3, reports the onset of migraines at the age of 40, describing them as right hemicranial and preceded by aura. The first episode of vertigo occurred at the age of 26 and has since become more frequent. She describes these vertigo episodes as periods of instability accompanied by intense neurovegetative symptoms lasting for days. Additionally, she experiences intermittent tinnitus without associated hypoacusis. Questionnaires scores: THI 22, GÜF 11, HADSa 15 and HADSd 8.

- The patient, II-4, is a 50-year-old woman with an incomplete phenotype, who began experiencing migraines in her twenties, with no history of aura. She has had episodes of dizziness, particularly motion-induced imbalance, sometimes accompanied by nausea. She has no history of tinnitus or hearing loss. Questionnaires scores: GÜF 13, HADSa 9 and HADSd 5.

- The III-1, the niece of the proband, is a healthy control. Questionnaires scores: GÜF 13, HADSa 6 and HADSd 1.

- The subject, III-2 is a probable VM. She is the niece of the proband, a 34-year-old woman who describes right hemicranial migraine with visual aura since she was twenty-two years old. Her audiological symptoms include hyperacusis, but she does not experience tinnitus or hearing loss. She describes her dizziness as a spinning sensation of objects that worsens with movement. At other times, she experiences episodes of instability lasting for hours. These symptoms are independent of each other. Questionnaires' scores: GÜF 9, HADSa 9 and HADSd 0.

- The patient III-4 is the proband's son. He is a 23-year-old male who has experienced migraines since childhood without visual aura. To date, he has not developed vestibular symptoms and has no associated audiological symptoms.

- The subject III-5 is a niece's proband; she is a 23-year-old female. Unfortunately, she did not complete the questionnaires. During the anamnesis, the patient reported migraines starting at age 19, without aura. She experiences phonophobia and photophobia, accompanied by nausea and vomiting during migraines. Vestibular symptoms appeared after the onset of migraines, described as minute-long episodes of unsteadiness. Audiological symptoms include hyperacusis, without tinnitus or hearing loss.

4.3.2.3   Family 3

This family is composed of individuals with definite VM (I-1 and II-1) and probable VM (III-1). Subject I-2, initially designated as a control, had to be excluded due to the poor quality of the exome sample.

- Subject I-1, the father of the proband, is a 71-year-old male. He has a history of hemicranial migraines with an early onset, beginning in childhood and lasting 24-48 hours, with regular neurological follow-up. He does not experience aura but reports phonophobia and phonophobia during migraines. The history of vertigo began 15 years ago at the age of 55, with episodes lasting minutes. He describes this vertigo as motion-induced dizziness. There are no reported symptoms of tinnitus or other associated symptoms. Questionnaires scores: THI 0, GÜF 13, Khalfa, 23, HADSa 3 and HADSd 3.

- The patient, II-1, is a 46-year-old woman who has experienced left hemicranial migraines since the age of 18. These migraines are accompanied by visual aura, phonophobia, and sonophobia, and can persist continuously for several days. The onset of vestibular symptoms occurred at the age of 25, seven years after the onset

of migraine episodes. She describes these symptoms as motion-induced dizziness, generating an oscillopsia sensation. Interestingly, migraines typically precede the onset of vestibular symptoms. The patient also experiences audiological symptoms, including hyperacusis, which worsens during migraines, and misophonia at 6000Hz. However, there is no reported hearing loss, sensation of ear fullness, or tinnitus. During a clinical examination conducted during an acute migraine episode, spontaneous nystagmus was detected. Questionnaires scores: GÜF 10, Khalfa 12, HADSa 3 and HADSd 3.

- Patient III-1, the proband's daughter, age 17, reported a history of migraine for the past 5 years, accompanied by intermittent and nonspecific episodes of dizziness. Unfortunately, detailed information from the questionnaires is not available.

4.3.2.4   Family 4

The last family consists of three individuals, two with definite MV and one with probable MV.

- The patient II-1, Patient I-1 is a 51-year-old woman with a history of pulsatile and hemicranial migraines. The migraine's onset is at the age of 16 and is preceded by a visual aura accompanied by phonophobia and photophobia. These migraines can persist for up to 2 days and have been correlated with menstruation, although their intensity has decreased with menopause. Vestibular symptoms first appeared at the age of 40, characterized by feelings of instability dizziness induced by head-motion. These symptoms are often accompanied by nausea and vomiting. Additionally, the patient does not report tinnitus, a sensation of fullness in the ears, and hearing loss as associated symptoms. Questionnaires scores: GÜF 8, Khalfa 19 HADSa 1 and HADSd 0.

- The proband, III-1, is a 30-year-old woman who reports a history of left retroocular and hemicranial migraine. These episodes are accompanied by aura, phonophobia, and photophobia. The age onset was 23 years old, 4 years before the development of the vestibular symptoms. She describes the vestibular symptoms as brief episodes of instability lasting seconds and accompanied by nausea and vomiting. Additionally, she reported intermittent tinnitus without any other auditory abnormalities. Questionnaires scores: THI 4, GÜF 5, Khalfa 12, HADSa 16 and HADSd 6.

- The subject III-2 is a 28-year-old woman who reports a history of classic migraine without aura since the age of 18, which can persist for days. The vestibular symptoms are unspecific, characterized by episodes of instability induced by movement of short

duration, without a close relationship between the two sets of symptoms. As auditory symptoms, this patient refers bilateral intermittent tinnitus. Questionnaires scores were: THI 10, GÜF 1, Khalfa 8, HADSa 6 and HADSd 6.

Table 20. Clinical characteristics of families with MV and summary of questionnaire scores

	Vestibular Migraine		General			Migraine			Vestibular Symptoms			Audiological symptoms			Scores				
PC	Status	YOE	Proc	Age	Sex	Onset	Feature	Aura	Onset	Type	Duration	Tinnitus	Hyperacusis	HL	THI	GÜF	HADSa	HADSd	Khalfa
Family 1																			
I-1	Probable	20	Sp	65	F	45	Decrease with menopause	No	45	Spinning/ MID		Permanent	No	SNHL	-	0	0	0	0
II-1	Definite	3	Sp	43	F	16	Menst-related	Visual	39	MID		Intermittent	No	No	4	7	18	6	
II-2	Proband	4	Sp	38	F	32		Visual	34	DHM	Hours	Permanent	Yes	No	6	3	8	4	
III-1	Only Migraine		Sp	8	M	3									-	-	-		-
Family 2																			
I-1	Only Migraine		Sp	89	F										-	-	-		-
II-1	Definite	20	Sp	62	F	25	Decrease with menopause	No	40	MID		Intermittent	Yes	SNHL	10	13	11	0	
II-2	Only Migraine		Sp	54	F	20	Menst-related	No				No	No	No	-	2	26	8	
II-3	Proband	10	Sp	52	F	40		Visual	26	Imbalance -NVS	Days	Intermittent	Yes	No	-	11	15	8	
II-4	Probable		Sp	50	F	22		No		MID-NVS		No	No	No	-	13	9	5	
III-1	Control		Sp	39	F										-	-	-		-
III-2	Probable		Sp	34	F	22		Visual		Spinning	Hours	No	Yes	No	-	9	9	0	
III-3	Probable		Sp	24	F														
III-4	Only Migraine		Sp	23	M														
III-5	Definite	3	Sp	23	F	19		Visual	20	MID	Minutes	No	Yes	No					
III-6	Definite		Sp	24	F														
Family 3																			
I-1	Definite	15	It	71	M	17		No	56	MID	Minutes	No	Yes	No	0	13	3	3	23

PC	VM	YOE	Proc	Age	Sex	Migraine onset	Feature	Aura	Vestibular onset	Type	Duration	Tinnitus	Hyperacusis	HL					
II-1	Proband	20	It	46	F	18	Spontaneus nystagmus	Visual	25	MID/oscillopsia		No	Yes	No	-	10	3	3	12
III-1	Probable		It	17	F	12			Ns	MID		No	No		-	-	-	-	-
Family 4																			
II-1	Definite	10	It	51	F	16	Menst-related	Visual	40	DHM-NVS		No	No	No	-	8	1	0	19
III-1	Proband	3	It	30	F	23		Visual	27	MID-NVS	Seconds	Intermittent	No	No	4	5	16	6	12
III-2	Probable		It	28	F	18		No		MID	Minutes	Intermittent	No	No	10	1	6	6	8

Information in table 20 include Pedigree familiar code (PC); VM column include Status of individuals referring to VM (definite VM, probable VM, only clinical of migraine and control), Years of evolution (YOE). General column include precedence (Proc.), Sp-Spain and It- Italy, Age in years and Sex (F-Female and M-Male). Migraine column include: onset, age onset of migraine; Feature of the migraine and its related with menstruation, Aura (if the migraine has an aura and what type of aura it is). Vestibular symptoms column included: onset, age onset of vestibular symptoms, Type, describe the characteristic of dizziness (MID- movement-induced dizziness, DHM- dizziness head-motion induced, NSV- neurovegetative symptoms); Duration of vestibular symptoms (seconds, minutes, hours, days). Audiological symptoms column included: Tinnitus classified in permanent and intermittent; Hyperacusis ("Yes" or "no" according to the patient's anamnesis) and HL (hearing loss) according presence or absence and type of hypoacusia (SNHL- Sensorineural hearing loss). Score column included the different scores of the questionnaires.

ND: non-described.

4.3.3 Audiological evaluation

In family 1, it was observed that subject I-1 exhibited SNHL in the higher frequencies but retained hearing in the lower and middle frequencies, consistent with presbycusis and compatible with the natural aging of the auditory pathway. Subjects II-1 and II-2 preserved their hearing; however, in the case of Subject II-1, it was only possible to study hearing up to the frequency of 8000 Hz (Figure 17).
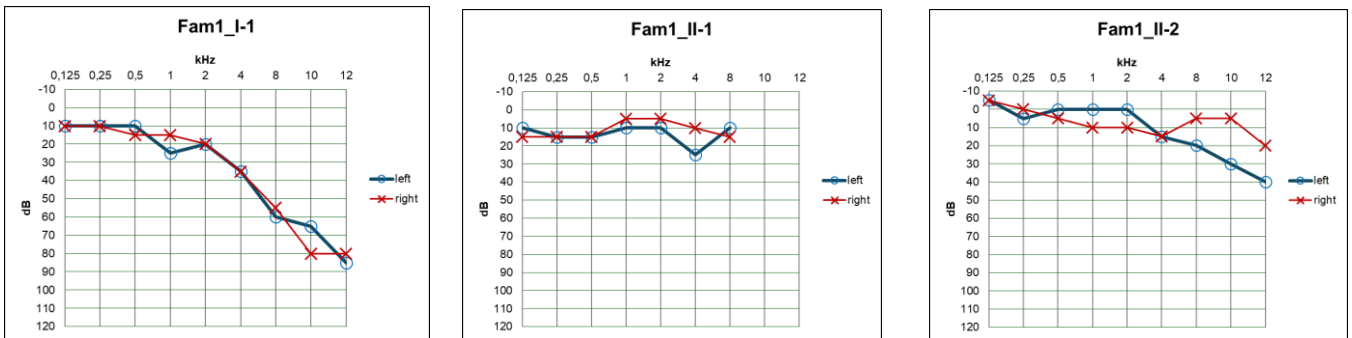
Figure 17. Shows the audiogram of the family 1, Subjects I-1 and II-2 were evaluated up to a frequency of 12 kHz, while subject II-1 could only be studied up to a frequency of 8 kHz.

Unfortunately, in family 2, the audiogram of the proband subject was only possible to obtain. The subject maintained normal hearing up to the high frequencies, where it began to decline (Figure 18).
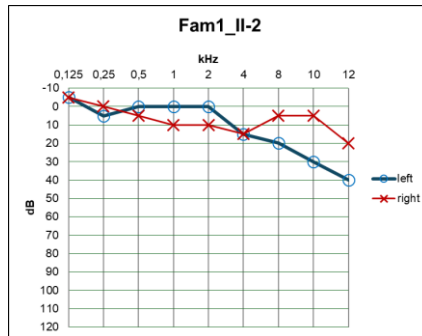
Figure 18. Shows the audiogram of family 2 proband, II-1.

The audiometries of the subjects in Family 3 were presented (Figure 19). They showed normal hearing at all frequencies except for the highest frequencies (> 10000 Hz).
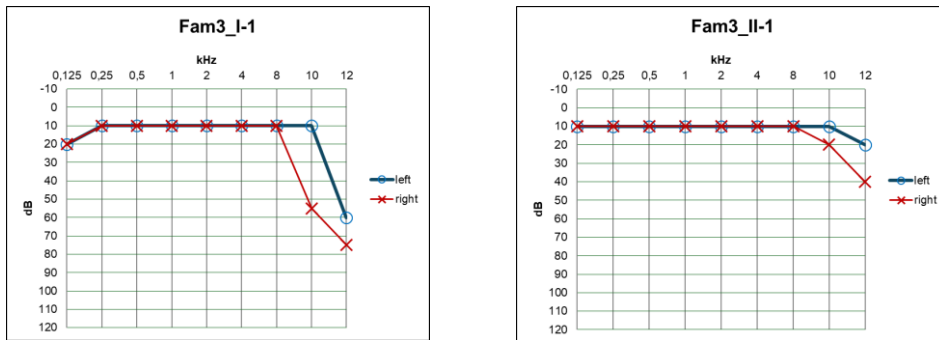
Figure 19. Shows the audiogram of family 3. Subjects I-1 and II-1 were evaluated up to a frequency of 12 kHz.

Finally, in family 4, it was observed that Subject II-1 presented mild pantonal hypoacusis, while Subject III-1 exhibited acoustic trauma at 8000 Hz, with the rest of the hearing within normal limits.
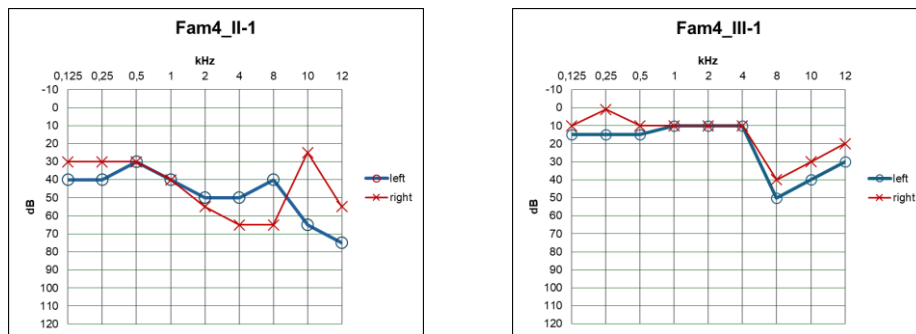
Figure 20. Shows the audiogram of the Family 4. Subjects II-1 and III-1 were evaluated up to a frequency of 12 kHz,

4.3.4   Candidate genes for Familial Vestibular Migraine in family 1

Various filters were applied to the total genes identified in Family 1 to obtain the list of candidate genes. Initially, 57,503 variants were detected, of which 29,010 were shared by the three sequenced family members.

To refine the list of variants shared by all three family members, common variants with a MAF > 0.1 in public databases, including gnomAD global, gnomAD NFE (Non-Finnish European), and CSVS (Collaborative Spanish Variant Service), were filtered out, resulting in 545 variants. Next, variants were filtered by impact, excluding those classified as Low and Modifier impact, reducing the list to 149 variants. Further filtering by CADD value, excluding those with a CADD < 20 and those found in the Otoscope database (which includes hearing loss genes), resulted in a final list of 57 variants for Family 1.

Once the filtering process was completed, the resulting variants were examined for their expression in various tissues using public databases, such as, GTEx, gEAR, SHIELD and Friedman's study. The primary focus was on their expression in brain tissues, the adult human inner ear, and the ear of the mouse. This comprehensive analysis aimed to identify variants not only present in the affected family members but also expressed in relevant tissues. Finally, 47 variants of 47 genes were expressed in these tissues, and Table 21 provides an overview of the final variants identified for Family 1.

4.3.5 Candidate genes for Familial Vestibular Migraine in family 2

In family 2, the sequencing of 6 individuals identified a total of 83,748 variants, with 19,141 shared by all members. Variants not expressed in the healthy control family member (Fam2_III-1) were filtered out, resulting in 1,199 variants. No variants of known genes for FHM and episodic ataxia were present. Filtering by MAF < 0.1 in gnomAD, gnomAD NFE, and CSVS reduced the number to 56 variants, of which only three had an impact of High or Moderate. None of these three variants had a CADD > 20.

After assessing their expression, according to the consulted databases, 1 variant was excluded due to lack of expression in brain or inner ear tissues, resulting in 2 variants (Table 22).

4.3.6 Candidate genes for Familial Vestibular Migraine in family 3

In family 3, after sequencing the family members, a total of 59,868 variants were obtained, of which 25,662 were common to the three individuals in the family. Filtering variants from public databases gnomAD global and gnomAD NFE with MAF < 0.1 resulted in 92 variants. Further filtering by impact, excluding those labeled as Low and Modifier, and by a CADD score > 20, resulted in 24 variants, further narrowed down to 12.

Analysis of these 12 variants through public databases such as GTEx, gEAR, SHIELD, and Friedman's study excluded one variant, resulting in a final count of 11 variants from 11 different genes (Table 23).

4.3.7 Candidate genes for Familial Vestibular Migraine in family 4

In family 4, WGS identified 58,338 variants, with 29,191 common variants for all the members.

Filtering variants from public databases, such as gnomAD global and gnomAD_NFE, with MAF < 0.1, resulted in 864 variants. Further filtering by impact, excluding those

labeled as Low and Modifier, and by a CADD score > 20, reduced this to 203 variants and 95, respectively.

The analysis of these 95 variants using public databases such as GTEx, gEAR, SHIELD, and the Friedman study excluded ten variants, leaving a final 85 variants from 84 genes. Two mutations were found in the *ANK3 gene* (Table 24).

This thorough filtering process aimed to narrow down the potential candidate genes that might be relevant to the VM phenotype observed in this family, focusing on variants with higher predicted pathogenicity and excluding common or less impactful variants.

Figure 21 shows the candidate variants in each family. It illustrates the reduction in the number of variants as the filters are applied.
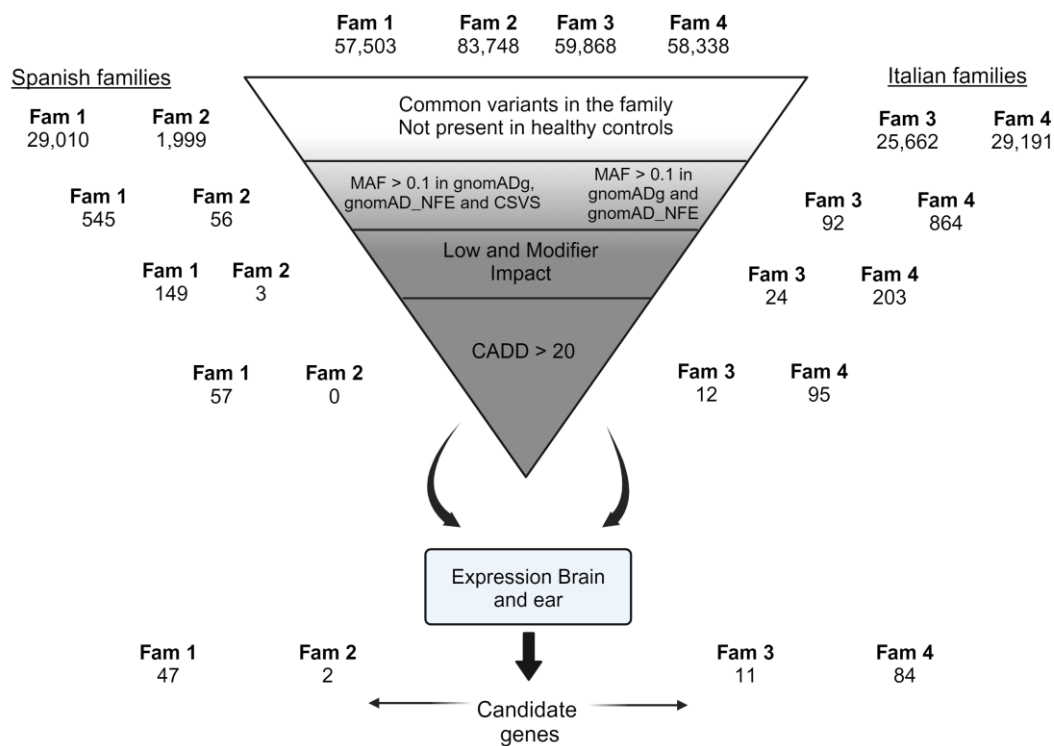
Figure 21. Summarizes the number of variants obtained in each family after applying the different filters. The lower part shows the genes that have been prioritized for each family. *Created with BioRender.com*

4.3.8   FHM and TRPM7 variants and distribution in families

We also wanted to know if any of the genes associated with FHM or Episodic Ataxia had variants in our families. Thus, we searched specifically for *CACNA1A*, *ATP1A2*, *SCN1A*, *KCNA1*, *CACNB4*, and *SLC1A3*. Additionally, we searched for variants in the TRPM7 gene. Several mutations were identified (table 25) but were discarded after applying subsequ ent filters, mainly MAF, CADD score, or due to their low or modifier impact.

Table 21. VM Family 1 candidate genes

Variant	ID	Gene	Consequence	CADD	AF global	AF NFE	CSVS	Fam 1 II-2	Fam 1 I-1	Fam 1 II-1
chr1:27361788G>A	rs11247641	*MAP3K6*	Missense	22.8	1.24E-02	2.35E-04	1.00E-03	0/1	0/1	0/1
chr1:167435412T>G	rs766541481	*CD247*	Missense	32	6.57E-03	0	0	0/1	0/1	0/1
chr1:236574683A>G	rs79225714	*HEATR1*	Missense	20.8	2.46E-02	8.12E-03	1.60E-02	0/1	0/1	0/1
chr2:172491072G>A	rs61737182	*ITGA6*	Missense	20.7	5.10E-03	7.47E-03	1.30E-02	0/1	0/1	0/1
chr3:15677542T>C	rs34208188	*ANKRD28*	Missense	23	2.89E-02	3.95E-02	3.90E-02	0/1	0/1	0/1
chr3:33132671C>T	rs115198029	*CRTAP*	Missense	24.3	2.47E-03	3.67E-03	1.00E-02	0/1	0/1	0/1
chr4:40152845G>A	rs113404449	*N4BP2*	Missense	29.3	2.19E-02	3.18E-02	4.30E-02	0/1	0/1	0/1
chr4:185418466T>C	rs61735474	*UFSP2*	Missense	23.7	1.82E-02	2.40E-02	3.30E-02	0/1	0/1	0/1
chr5:64724489T>G	rs80170948	*SREK1IP1*	Missense	22.4	2.80E-02	4.19E-02	3.40E-02	0/1	0/1	0/1
chr5:81330515C>G	rs148630854	*ACOT12*	Missense	22.5	4.32E-03	7.94E-04	3.00E-03	0/1	0/1	0/1
chr6:25537960C>A	rs12527029	*CARMIL1*	Missense	23.4	2.18E-02	4.18E-03	2.20E-02	0/1	0/1	0/1
chr6:30625708C>G	rs34315095	*MRPS18B*	Missense	24.8	4.42E-02	1.77E-02	3.60E-02	0/1	0/1	0/1
chr6:43339661G>A	rs145629243	*ZNF318*	Missense	24.1	7.27E-03	1.18E-02	5.00E-03	0/1	0/1	0/1
chr6:157110487C>T	rs754080772	*ARID1B*	Missense	25.8	5.26E-02	1.47E-05	0	0/1	0/1	0/1
chr7:105508146G>A	rs61741425	*PUS7*	Missense	23.8	1.44E-02	2.14E-02	2.20E-02	0/1	0/1	0/1
chr8:116852066A>C	rs144953114	*RAD21*	Missense	29	3.35E-04	5.73E-04	0	0/1	0/1	0/1
chr8:144791484T>C	rs11539893	*RPL8*	Missense	20.4	2.30E-02	3.28E-02	3.10E-02	0/1	0/1	0/1
chr9:33467731C>T	rs61995751	*NOL6*	Missense	23.3	1.39E-02	2.22E-02	1.00E-02	0/1	0/1	0/1
chr9:77405958C>A	rs117983287	*VPS13A*	Missense	21	8.50E-03	1.34E-02	1.60E-02	0/1	0/1	0/1
chr9:114626987T>C	rs139126699	*TMEM268*	Missense	21.4	1.10E-03	1.73E-03	5.00E-03	0/1	0/1	0/1
chr9:125915818C>T	rs145687528	*PBX3*	Missense	24.3	6.17E-03	8.15E-03	8.00E-03	0/1	0/1	0/1
chr10:70124080C>A	rs41277978	*AIFM2*	Missense	23.6	4.10E-03	6.84E-03	7.00E-03	0/1	0/1	0/1
chr10:71295850G>A	rs61749231	*UNC5B*	Missense	20.2	4.92E-03	6.61E-03	1.20E-02	0/1	0/1	0/1

chr10:73136789T>A	rs149666428	*ECD*	Missense	21	7.76E-04	1.35E-03	2.00E-03	0/1	0/1	0/1
chr10:114286037G>A	rs79009215	*VWA2*	Missense	23.5	9.22E-03	1.24E-02	1.50E-02	0/1	0/1	0/1
chr10:122237455G>A	rs61753077	*TACC2*	Missense	25.8	2.16E-02	3.61E-02	4.10E-02	0/1	0/1	0/1
chr10:132208072C>T	rs55812591	*STK32C*	Missense	20.4	2.18E-02	1.69E-02	2.10E-02	0/1	0/1	0/1
chr12:2858912G>A	rs28919870	*FOXM1*	Missense	24.6	6.25E-03	9.80E-03	2.00E-02	0/1	0/1	0/1
chr12:32896656C>T	rs143004808	*PKP2*	Missense	26.6	7.07E-03	9.11E-03	1.00E-02	0/1	0/1	0/1
chr13:98213265C>T	rs9300466	*FARP1*	Missense	23.4	1.11E-02	2.79E-04	1.00E-03	0/1	0/1	1/1
chr13:98712530G>T	rs779077196	*SLC15A1*	Missense	26	3.94E-02	0	0	0/1	0/1	0/1
chr14:93183368T>C	rs74075220	*MOAP1*	Missense	22.3	5.93E-03	9.99E-04	3.00E-03	0/1	0/1	0/1
chr15:29733199C>T	rs200912609	*TJP1*	Missense	24.4	9.60E-04	1.69E-03	0	0/1	0/1	0/1
chr15:40856790G>A	rs142240169	*SPINT1*	Missense	24.4	5.37E-03	9.44E-03	6.00E-03	0/1	0/1	0/1
chr17:73456015C>T	rs374727187	*SDK2*	Missense	23.8	3.68E-04	7.50E-04	0	0/1	0/1	0/1
chr17:75611622C>G	rs768408419	*MYO15B*	Missense	24.7	2.17E-04	2.21E-04	1.00E-03	0/1	0/1	0/1
chr17:75742728C>T	rs145976111	*ITGB4*	Missense	30	2.31E-03	3.25E-03	4.00E-03	0/1	0/1	0/1
chr17:81231872G>A	rs61745844	*TEPSIN*	Missense	23.1	6.48E-03	9.37E-03	1.20E-02	0/1	0/1	0/1
chr19:32692087C>T	rs200386314	*NUDT19*	Missense	32	9.06E-03	1.50E-02	8.00E-03	0/1	0/1	0/1
chr19:33094219G>T	rs369545403	*GPATCH1*	Missense	25.7	1.31E-04	1.62E-04	0	0/1	0/1	0/1
chr19:34685134A>G	rs116552744	*ZNF302*	Missense	21.3	6.21E-03	9.26E-04	6.00E-03	0/1	0/1	0/1
chr19:35155578C>T	rs781491924	*FXYD5*	Missense	22.5	3.29E-02	2.94E-02	2.00E-03	0/1	0/1	0/1
chr19:39915630G>A	rs147566832	*FCGBP*	Missense	20.7	2.10E-02	8.70E-03	1.00E-02	0/1	0/1	0/1
chr19:40580817C>G	rs137860505	*SHKBP1*	Missense	26.9	1.09E-03	6.76E-04	1.00E-03	0/1	0/1	0/1
chr19:40800745C>T	rs61750953	*EGLN2*	Missense	21.3	1.38E-02	1.77E-02	3.80E-02	0/1	0/1	0/1
chr19:43494791G>A	rs543150798	*PHLDB3*	Missense	24.9	2.63E-02	4.41E-02	0	0/1	0/1	0/1
chr20:6077187C>T	rs376128513	*FERMT1*	Missense	23.8	3.29E-02	4.41E-05	1.00E-03	0/1	0/1	0/1

Table 22. Candidate genes as the origin of MV in family 2

Variant	ID	Gene	Consequence	CADD	AF global	AF NFE	CSVS	Fam 2 II-1	Fam 2 III-5	Fam 2 II-4	Fam 2 III-2	Fam 2 II-3	Fam 2 III-1
chr16:88433940G>A	rs13334190	*ZNF469*	Missense	0.026	9,07E-02	5,86E-02	6,10E-02	0/1	1/1	1/1	0/1	0/1	./.
chr19:42358883A>G	rs1206038	*MEGF8*	Missense	0.943	8,68E-02	5,47E-02	6,90E-02	0/1	0/1	0/1	0/1	0/1	./.

Table 23. Candidate genes as origin of VM in family 3

Variant	ID	Gene	Consequence	CADD	AF global	AF NFE	Fam 3 III-1	Fam 3 II-1	Fam 3 I-1
chr1:46035072G>C	rs3737737	*MAST2*	Missense	22.6	2.91E-02	2.35E-02	0/1	0/1	0/1
chr2:160137782C>T	rs61737764	*ITGB6*	Missense	25	4.30E-03	5.73E-03	0/1	0/1	0/1
chr6:29632327G>A	rs1805056	*GABBR1*	Missense	24.4	4.44E-02	4.83E-02	0/1	1/1	0/1
chr8:122953845G>A	rs3802264	*ZHX2*	Missense	22	4.29E-02	3.10E-02	0/1	1/1	0/1
chr11:74057508C>T	rs12419308	*C2CD3*	Missense	23.5	4.69E-02	2.68E-02	0/1	0/1	0/1
chr11:74336698T>A	rs36014178	*PGM2L1*	Missense	23.7	3.59E-02	3.93E-02	0/1	0/1	0/1
chr13:37569335A>G	rs75157793	*POSTN*	Missense	22.6	7.11E-03	9.83E-03	0/1	0/1	0/1
chr17:36953122G>A	rs28656116	*AATF*	Missense	24.1	1.39E-02	3.38E-04	0/1	1/1	0/1
chr17:47975517G>A	rs61741125	*CDK5RAP3*	Missense	25	2.90E-02	4.08E-02	0/1	0/1	0/1
chr17:56499041G>C	rs61753934	*ANKFN1*	Missense	27	3.39E-02	2.06E-02	0/1	0/1	0/1
chr19:1506032G>A	rs144153954	*ADAMTSL5*	Missense	23.8	5.18E-03	7.75E-03	0/1	1/1	0/1

Table 24. Candidate genes as origin of MV in family 4

Variant	ID	Gene	Consequence	CADD	AF global	AF NFE	Fam 4 III-1	Fam 4 III-2	Fam 4 II-1
chr1:1044368A>T	rs113288277	*AGRN*	Missense	28.6	2.95E-02	4.48E-02	0/1	0/1	0/1
chr1:1311071G>A	rs140444081	*PUSL1*	Missense and splice region variant	32	1.18E-04	2.50E-04	0/1	0/1	0/1
chr1:9128930A>G	rs114206792	*GPR157*	Missense	23.6	2.41E-02	1.26E-02	0/1	0/1	0/1
chr1:16055706G>A	rs143663847	*CLCNKB*	Missense	24.9	1.49E-02	1.12E-02	0/1	0/1	0/1
chr1:150557533C>T	rs115937511	*ADAMTSL4*	Missense	24.8	2.78E-03	7.21E-04	0/1	0/1	0/1
chr1:201325782C>T	rs61818256	*PKP1*	Missense	28.5	1.45E-02	1.95E-02	0/1	0/1	0/1
chr1:232807728C>A	rs200746267	*MAP10*	Missense	22.2	3.55E-04	5.29E-04	0/1	0/1	0/1
chr1:232986138T>C	rs41304133	*PCNX2*	Missense	25.4	1.62E-02	2.40E-02	0/1	0/1	0/1
chr2:24164308GC>G	.	*FAM228B*	Frameshift	27	3.18E-02	2.79E-02	0/1	0/1	1/1
chr2:36773813C>G	rs140104536	*VIT*	Stop gained	35	8.07E-03	1.22E-02	0/1	0/1	0/1
chr2:43762386C>G	rs76859622	*PLEKHH2*	Missense	23.4	2.27E-02	3.19E-02	0/1	0/1	0/1
chr2:84870466C>T	rs1863772	*TRABD2A*	Missense	20.7	2.58E-02	3.85E-02	0/1	0/1	0/1
chr3:57098007G>A	rs61742267	*IL17RD*	Missense	22.6	1.39E-02	2.06E-02	1/1	0/1	0/1
chr4:86762995G>T	rs201633051	*PTPN13*	Missense	25	3.69E-03	5.55E-03	0/1	0/1	0/1
chr4:94455353A>G	rs75841704	*PDLIM5*	Missense	29.5	1.65E-02	2.56E-02	0/1	0/1	0/1
chr4:121670000C>T	rs145513784	*ANXA5*	Missense	27.4	7.11E-04	5.44E-04	0/1	0/1	0/1
chr4:158721424G>A	rs2070631	*PPID*	Missense	26.2	2.67E-02	2.87E-02	0/1	0/1	0/1
chr4:185418466T>C	rs61735474	*UFSP2*	Missense	23.7	1.82E-02	2.40E-02	0/1	0/1	0/1
chr5:95654174A>C	rs74672963	*RFESD*	Missense	21.9	4.79E-02	3.66E-02	0/1	0/1	0/1
chr5:123390074G>A	rs61747983	*CEP120*	Missense	21.8	1.58E-02	1.47E-04	0/1	0/1	0/1
chr5:139848111C>T	rs149093144	*NRG2*	Missense	25.3	6.29E-03	6.46E-03	0/1	0/1	0/1
chr5:140927719C>A	rs144571902	*PCDHAC1*	Missense	23.4	1.38E-04	2.21E-04	0/1	0/1	0/1
chr5:160634558C>A	rs149397148	*ATP10B*	Missense	26.4	1.23E-02	1.97E-02	0/1	0/1	0/1

Position	rsID	Gene	Consequence							
chr6:31736288GTTC>G	rs147567766	**CLIC1**	Inframe deletion	22.1	3.00E-02	4.04E-02	0/1	0/1	0/1
chr6:35797266A>G	rs2766597	**CLPS**	Missense	25.6	3.75E-02	1.43E-02	0/1	0/1	0/1
chr6:43516794G>A	rs2231763	**YIPF3**	Missense	22.4	2.51E-02	3.97E-02	0/1	0/1	0/1
chr6:46167472A>G	.	**ENPP5**	Missense	23.6	6.57E-03	1.47E-05	0/1	0/1	0/1
chr6:89153050G>T	rs377565760	**PM20D2**	Missense	26.7	8.54E-02	1.32E-04	0/1	0/1	0/1
chr6:142166242A>G	rs752875745	**VTA1**	Missense	20.3	1.31E-02	1.47E-05	0/1	0/1	0/1
chr7:43608339G>A	rs77026432	**STK17A**	Missense	24.1	6.71E-03	4.41E-02	0/1	0/1	0/1
chr7:100621008C>T	rs41295942	**TFR2**	Missense	23.1	2.12E-02	3.30E-02	0/1	0/1	0/1
chr7:151024396T>TC	rs766474019	**ATG9B**	Frameshift	22.6	8.09E-04	5.20E-04	0/1	0/1	0/1
chr10:7248643C>T	rs117741182	**SFMBT2**	Missense	23	2.67E-03	3.44E-03	0/1	0/1	0/1
chr10:24536832G>A	rs145406190	**KIAA1217**	Missense	28.9	2.24E-04	2.50E-04	0/1	0/1	0/1
chr10:60069791G>C	rs148109897	**ANK3**	Missense	23.4	2.50E-04	4.56E-04	0/1	0/1	0/1
chr10:60073656A>G	rs148904927	**ANK3**	Missense	26.4	4.71E-03	6.56E-03	0/1	0/1	0/1
chr10:69227304A>G	rs10823320	**HKDC1**	Missense	23.2	3.02E-02	3.97E-02	0/1	0/1	0/1
chr10:70600631G>A	rs35947132	**PRF1**	Missense	24.8	2.87E-02	4.48E-02	0/1	0/1	1/1
chr10:72341053C>T	rs11552373	**DNAJB12**	Missense	25.7	2.27E-03	3.75E-03	0/1	0/1	0/1
chr10:88764578C>T	rs41284088	**LIPN**	Missense	22.9	1.82E-02	2.64E-02	0/1	0/1	0/1
chr10:101534803A>G	rs141008374	**BTRC**	Missense	22.3	6.84E-03	4.41E-05	0/1	0/1	0/1
chr10:110784367C>T	rs189569984	**RBM20**	Missense	25.8	5.38E-03	7.82E-03	0/1	0/1	0/1
chr11:66690210C>T	rs35532855	**SPTBN2**	Missense	22.5	1.59E-02	2.49E-02	0/1	0/1	0/1
chr11:83280221C>T	rs61747435	**CCDC90B**	Missense	26.9	4.54E-03	6.71E-03	0/1	0/1	0/1
chr12:47793546G>A	rs77196719	**HDAC7**	Missense	23.2	7.75E-03	1.22E-02	0/1	0/1	0/1
chr12:54508480G>A	rs149360088	**NCKAP1L**	Missense	23.2	1.26E-03	2.35E-03	0/1	0/1	0/1
chr15:42448362T>C	rs142908361	**ZNF106**	Missense	23.1	1.39E-03	2.15E-03	0/1	0/1	0/1
chr15:44615364T>C	rs111347025	**SPG11**	Missense and splice region variant	23	9.46E-03	1.34E-02	0/1	0/1	0/1

chr15:52343197T>A	rs61731219	*MYO5A*	Missense and splice region variant	23.2	3.39E-02	4.85E-02	0/1	0/1	0/1
chr15:90225710C>T	rs115820812	*SEMA4B*	Missense	26.5	2.12E-02	1.47E-03	0/1	0/1	0/1
chr15:90241854CG>C	rs577983969	*GDPGP1*	Frameshift	33	6.11E-04	2.94E-05	0/1	0/1	0/1
chr15:90749678A>G	rs28384988	*BLM*	Missense	22.6	4.01E-03	5.88E-02	0/1	0/1	0/1
chr15:90936432G>A	rs147481345	*UNC45A*	Missense	26	6.18E-04	0	0/1	0/1	0/1
chr16:18854886C>G	rs17731779	*SMG1*	Missense	20.7	1.55E-02	2.16E-02	0/1	0/1	0/1
chr16:19037931A>T	rs55796412	*TMC7*	Missense	23	8.74E-03	1.28E-02	0/1	0/1	0/1
chr16:68255978G>A	rs752888337	*PLA2G15*	Missense	24.4	3.94E-02	8.82E-02	0/1	0/1	0/1
chr16:69136838G>T	rs144369314	*UTP4*	Missense	28.9	2.27E-03	3.48E-03	0/1	0/1	0/1
chr16:72958768C>G	rs2073852	*ZFHX3*	Missense	21.7	1.63E-02	1.04E-02	0/1	0/1	0/1
chr17:42798090G>C	rs150778923	*COA3*	Missense	25.9	2.37E-04	3.68E-04	0/1	0/1	0/1
chr17:47975517G>A	rs61741125	*CDK5RAP3*	Missense	25	2.90E-02	4.08E-02	0/1	0/1	0/1
chr17:48610773G>A	rs79247310	*HOXB7*	Missense	22.9	4.75E-02	3.34E-02	0/1	0/1	0/1
chr17:50188134C>T	rs1800215	*COL1A1*	Missense	20.9	2.56E-02	1.66E-02	0/1	0/1	0/1
chr17:56466411G>A	rs149688029	*ANKFN1*	Missense	25.1	6.51E-04	8.23E-04	0/1	0/1	0/1
chr17:69016309G>A	rs73370041	*ABCA9*	Missense	23.8	1.39E-02	1.06E-03	0/1	0/1	0/1
chr17:69277786C>A	rs142165698	*ABCA5*	Missense	25.9	1.01E-03	1.32E-04	0/1	0/1	0/1
chr17:78569473C>T	rs144524797	*DNAH17*	Missense	22.6	6.77E-04	3.09E-04	0/1	0/1	0/1
chr17:81528796C>T	rs782187587	*FSCN2*	Missense	23.9	3.94E-02	2.94E-05	0/1	0/1	0/1
chr17:81696724G>A	rs533048809	*HGS*	Missense	23.2	6.57E-06	1.47E-05	0/1	0/1	0/1
chr18:33746048T>G	rs144534810	*ASXL3*	Missense	25.2	6.68E-03	1.05E-02	0/1	0/1	0/1
chr18:45668426G>A	rs148964498	*SLC14A2*	Missense	26.6	4.53E-04	5.29E-04	0/1	0/1	0/1
chr18:78992599C>A	rs201351909	*SALL3*	Missense	23.7	7.34E-02	1.64E-04	0/1	0/1	0/1
chr19:2039765C>T	rs142610986	*MKNK2*	Missense	24	2.37E-04	2.65E-04	0/1	0/1	0/1
chr19:3752603G>A	rs147130540	*APBA3*	Missense	26.4	1.82E-02	2.52E-02	0/1	0/1	0/1
chr19:5223007G>A	rs61729778	*PTPRS*	Missense	23.6	2.56E-02	3.55E-02	0/1	0/1	0/1

chr19:7899597G>A	rs45584934	**LRRC8E**	Missense	25.3	1.88E-03	2.88E-03	0/1	0/1	0/1
chr19:8081044C>T	rs3848570	**FBN3**	Missense	22.2	3.35E-02	4.85E-02	0/1	0/1	0/1
chr19:19545747A>G	rs147526697	**CILP2**	Missense	23	1.25E-03	1.25E-03	0/1	0/1	0/1
chr19:21727323G>T	rs138292237	**ZNF100**	Stop gained	32	8.06E-03	1.17E-02	0/1	0/1	0/1
chr19:29613964G>T	rs61731483	**POP4**	Missense	23.3	1.51E-02	2.40E-02	0/1	0/1	0/1
chr19:35560431G>T	rs139075511	**ATP4A**	Missense	23.9	7.98E-03	1.20E-02	0/1	0/1	0/1
chr19:51006545C>T	.	**KLK9**	Missense	24.6	6.59E-03	1.47E-02	0/1	1/1	0/1
chr19:54463277C>A	rs149393351	**LENG9**	Missense	25	7.68E-03	1.28E-02	0/1	0/1	0/1
chr21:33403580C>T	.	**IFNGR2**	Missense	22.8	1.21E-03	1.48E-03	0/1	0/1	0/1
chr22:17818166G>A	rs373213687	**MICAL3**	Missense	23.1	5.92E-02	2.94E-02	0/1	0/1	0/1
chr22:37932638G>A	rs140512229	**MICALL1**	Missense	25	1.31E-02	1.47E-05	0/1	0/1	0/1

Table 25. Genes related to Familial Hemiplegic Migraine and TRPM7 and distribution in families.

Variant	ID	Gene	Consequence	CADD	AF global	AF NFE	CSVS			
Family 1								Fam 1 II-2	Fam 1 I-1	Fam 1 II-1
chr2:166036278C>T	rs2298771	**SCN1A**	Missense	7.679	7.37E-01	6.83E-01	5.89E-01	0/1	0/1	1/1
chr2:166041354A>G	rs6432860	**SCN1A**	Synonymous Variant	0.736	7.38E-01	6.83E-01	5.85E-01	0/1	0/1	1/1
chr2:166046935T>C	rs7580482	**SCN1A**	Synonymous Variant	8.302	6.67E-01	6.82E-01	6.23E-01	0/1	0/1	1/1
chr12:4912062T>C	rs1048500	**KCNA1**	Synonymous Variant	10.76	4.71E-01	4.91E-01	5.19E-01	0/1	0/1	0/1
chr12:4912182G>C	rs2227910	**KCNA1**	Synonymous Variant	5.752	5.08E-01	5.11E-01	5.32E-01	0/1	0/1	0/1
chr12:4912818T>A	rs4766309	**KCNA1**	Synonymous Variant	9.094	7.53E-01	6.79E-01	6.98E-01	1/1	1/1	1/1

Variant	rsID	Gene	Type	Score	P1	P2	P3			
chr15:50574885G>A	rs473357	*TRPM7*	Synonymous Variant	6.845	5.90E-01	5.58E-01	6.23E-01	0/1	1/1	0/1
chr19:13208879A>G	rs16051	*CACNA1A*	Synonymous Variant	8.804	6.71E-01	6.23E-01	3.09E-01	0/1	1/1	0/1
Family 3								Fam 3 II-1	Fam 3 I-1	Fam 3 III-1
chr2:166036278C>T	rs2298771	*SCN1A*	Missense	7.679	7.37E-01	6.83E-01		1/1	1/1	0/1
chr2:166041354A>G	rs6432860	*SCN1A*	Synonymous Variant	0.736	7.38E-01	6.83E-01		1/1	1/1	0/1
chr2:166046935T>C	rs7580482	*SCN1A*	Synonymous Variant	8.302	6.67E-01	6.82E-01		1/1	1/1	0/1
chr19:13206676GTTA>G	rs143342886	*CACNA1A*	3 prime UTR variant	3.166	5.45E-01	4.44E-01		1/1	1/1	1/1
chr19:13207858CCTGCTG>C	rs16054	*CACNA1A*	Inframe Deletion	10.77	3.89E-01	3.27E-01		1/1	0/1	1/1
chr19:13298576C>T	rs16025	*CACNA1A*	Synonymous Variant	8.339	1.41E-01	1.51E-01		0/1	0/1	0/1
chr19:13298658T>A	rs16023	*CACNA1A*	Missense	22.5	1.34E-01	1.51E-01		0/1	0/1	0/1
chr19:13334394C>T	rs2248069	*CACNA1A*	Synonymous Variant	8.976	6.45E-01	6.84E-01		0/1	0/1	0/1
Family 4								Fam 4 III-2	Fam 4 III-1	Fam 4 II-1
chr2:166036278C>T	rs2298771	*SCN1A*	Missense	7.679	7.37E-01	6.83E-01		1/1	1/1	1/1
chr2:166041354A>G	rs6432860	*SCN1A*	Synonymous Variant	0.736	7.38E-01	6.83E-01		1/1	1/1	1/1
chr2:166046935T>C	rs7580482	*SCN1A*	Synonymous Variant	8.302	6.67E-01	6.82E-01		1/1	1/1	1/1
chr12:4912062T>C	rs1048500	*KCNA1*	Synonymous Variant	10.76	4.71E-01	4.91E-01		1/1	1/1	1/1
chr12:4912182G>C	rs2227910	*KCNA1*	Synonymous Variant	5.752	5.08E-01	5.11E-01		1/1	1/1	1/1
chr12:4912818T>A	rs4766309	*KCNA1*	Synonymous Variant	9.094	7.53E-01	6.79E-01		1/1	1/1	1/1

5 DISCUSSION

This thesis offers a unique and targeted analysis of our patient sample, divided into three major sections: tinnitus cohort, VM cohort, and familial VM cohort, following each specific aim. This thesis is based on the hypothesis that patients with VM exhibit specific psychoacoustic characteristics distinct from other disorders; in addition, we hypothesize that there is a genetic basis in familial cases of VM, which accounts for the occurrence of multiple cases within the same phenotype in the family. Throughout the development of the thesis, we have been studying the characteristics of these patients, ranging from common disorders such as tinnitus to the individual examination of families. Additionally, we propose a series of candidate genes that, according to the variants found, could contribute to the development of the VM phenotype in different families.

Our approach involved a comprehensive comparison and contrast of outcomes across varying demographic, clinical, and genetic variables. This allowed us to dissect the influences of different conditions on diverse patient groups, providing a more accurate and detailed analysis of our data. By doing so, we have made a valuable contribution to the field, enhancing our understanding of the clinical profile of VM.

5.1 Tinnitus Cohort

This first study aimed to investigate the clinical profile of Spanish patients suffering from chronic tinnitus, a condition that affects a significant portion of the population. We utilized the ESIT-SQ responses in two groups: outpatients and online participants. The ESIT-SQ is a comprehensive self-reported questionnaire that records information on various diseases, including hearing loss, anxiety/depression, and MD. It is an essential tool in our research and should be used as a screening instrument, with a clinical diagnosis required to confirm any disease or disorder. Despite its limitations, our study identified a set of characteristics commonly present in participants with tinnitus, which provides valuable insights into this complex condition.

The ESIT-SQ is a recently developed questionnaire by Genitsaridi et al., and its use in studies is still relatively limited[151]. This underscores the necessity for further research in this area. Studies are also needed comparing population characteristics between face-to-face and self-report questionnaires. However, the use of online questionnaires to study tinnitus has already been reported by Michiels et al. (2019). This study recorded some of the physical symptoms included in the ESIT-SQ, such as neck pain and headache, through an online questionnaire. The presence of neck pain was higher in this study, compared to our outpatient and online cohorts, but headache was reported in

fewer patients than in our sample[152,153]. These findings highlight the importance of conducting more comprehensive studies to fully understand the clinical profile of tinnitus.

Most individuals who responded to the online survey were patients with chronic tinnitus and MD, a chronic inner ear disorder defined by episodes of vertigo associated with tinnitus and SNHL[154,155]. Although this could be considered a bias for the online survey and should not be extended to the general population, these results may contribute to a better understanding of the tinnitus profile in MD. This study had the limitations of cross-sectional design; therefore, we could not rule out the possibility of reverse causality. Regardless, the analysis of two samples of individuals with tinnitus from the same population can help find differences in anonymous responses obtained online with the responses given by patients with confirmed chronic tinnitus at the hospital.

Regarding the characteristics of each group, the median age value was 44 (37 – 53) in the online sample and 55 (46 – 62) in outpatients. Few studies have investigated the effect of age on the responses obtained to assess comorbidities in patients with tinnitus. There are some studies where the average age was higher[156], although this research study analyzed the aging population and included patients over 45. However, Alsanosi[157], who studied the Saudi population, reported a lower average age. Our results showed differences between both groups regarding the education level. Both groups showed a high educational level; however, the level of education was significantly higher in the online population. One study showed a lower educational level in patients with tinnitus than healthy controls. However, the results were not statistically significant[156]. Another research showed an association between a lower level of education and tinnitus. This association was not confirmed after a multiple logistic regression analysis[157]. We could partially explain our findings through the characteristics of the online sample, consisting of individuals with higher knowledge of technology, which was not necessary in the outpatient sample.

The ESIT-SQ recorded information about otological disorders such as acoustic trauma, infections, or different types of hearing loss. Although we have not found any study with the same classification of otological disorders, Kim et al. described an association between abnormal tympanic membrane and tinnitus. However, they did not specify the origin of such tympanic alteration. Only a few otological diseases could eventually lead to tympanic membrane abnormalities[158]. Regardless, our results were not statistically significant.

In several studies, hearing loss has shown a significant association with tinnitus[58,159]. However, Mores et al. reported that hearing loss has little impact on perceived tinnitus[153].

Their results showed differences between intensity and the minimum masking level that could be explained by cochlear damage. In this regard, the ESIT-SQ could not distinguish the type of hearing loss, its laterality, or the hearing threshold. However, we observed differences in hearing impairment when comparing both groups, with higher rates of presbycusis in the outpatient cohort, although there were no statistically significant differences in our study.

In the online survey, vertigo was reported more frequently. This could be due to the higher prevalence of MD in this group. However, dizziness and vertigo are non-specific symptoms that can be associated with multiple causes, including vestibular, brain, or heart disorders. Additionally, headaches, sleep disorders, or stress could also be related to both symptoms[160].

Stress, depression, and anxiety are often associated with tinnitus, as shown in previous studies[161–164]. In our research, one-third of the participants in the online survey and a quarter of those in the outpatient cohort reported feeling anxious. A case-control study conducted by Ivansic found that patients with severe tinnitus were more likely to experience psychiatric disorders, such as depression and elevated somatization[165]. Respondents frequently reported experiencing sleep disorders, with a higher prevalence among outpatients compared to online participants. However, this difference was no longer significant after adjusting for sex and age. It is worth noting that previous studies have shown that women with tinnitus are more likely to experience sleep disorders[166].

We did not find a higher prevalence of temporomandibular joint disorders than the rates described in previous studies[167,168]. Some potential risk factors for tinnitus, such as hypertension, dyslipidemia, diabetes mellitus, stroke, angina, and myocardial infarction, have been proposed[58,159], but their association with tinnitus is controversial[159,169]. Our study compared the frequency of these risk factors in outpatients versus an online cohort. We found dyslipidemia to be more common among outpatients, but after adjusting for sex and age, the rates of hypertension and dyslipidemia were similar in both groups. Our study also found no differences in body mass index (BMI) between the two groups, with a rate of obesity of almost 20% in the outpatients and the online cohort. Some studies have reported that obesity may decrease the susceptibility to tinnitus[169], while others have reported a higher risk of tinnitus in those with a BMI > 30 kg/m2[58,158]. However, a BMI ≥30 kg/m2 could be associated with a higher rate of dyslipidemia and hypertension among the tinnitus group.

We observed differences in the prevalence of hyperacusis in both online and outpatient cohorts, affecting a significant percentage of survey respondents who had tinnitus.

Hyperacusis was more commonly reported in the online survey, possibly due to a higher proportion of patients with MD in this group. Studies have shown that hyperacusis is more frequent in patients with tinnitus than in those without it. It is also well-established that tinnitus frequently co-occurs with hyperacusis, particularly in severe cases[161,170]. Furthermore, both conditions may share a common pathophysiology[21].

There is a lack of studies focused on the characteristics of tinnitus, although multiple variables may explain its heterogeneity. Genitsaridi et al. conducted a systematic review to summarize the symptoms frequently recorded in studies and those that showed significant differences among different groups of patients. The most reported characteristics were tinnitus severity, hearing stage, age, and depressive symptoms[164].

The duration of tinnitus significantly differed between the two groups, with the online survey group reporting a higher duration. Other studies have also shown that patients consider tinnitus duration an important variable. However, these results were not compared with those of a control group[164].

Our analysis of MD patients found that tinnitus is associated with changes in hearing and triggers such as pressure changes. Patients with MD also showed a higher prevalence of hyperacusis and worry about tinnitus. These findings have been previously reported in MD[171]. Additionally, it has been observed that patients with MD have a higher prevalence of anxiety, as measured by questionnaires such as the Hospital Anxiety and Depression Scale (HADS). This increase in psychiatric symptoms could be because our patients with MD were recruited through a volunteer questionnaire and are more aware of their disease due to its severity. Furthermore, HADS scores positively correlated with other quality-of-life questionnaires related to tinnitus, such as the Tinnitus Handicap Inventory (THI) or the visual analog scale on tinnitus annoyance[172].

There are controversies regarding the effectiveness of various treatments for tinnitus. Several studies have shown the impact of multiple treatments on tinnitus, but the results are limited due to the variability of the data[173]. Niemann et al. studied the clinical profiles and psychoacoustic features of men and women suffering from tinnitus. They discovered that there were gender differences in the effectiveness of treatment in terms of tinnitus-related distress and severity of depression[166]. Our research was not segregated by gender, but we observed distinctions between outpatients and online participants in the study sample. Genitsaridi et al. also reported gender-based disparities in the effectiveness of therapy. Further studies may be beneficial to confirm these findings and develop more effective treatment approaches for tinnitus[164].

A limited amount of research is available on the psychoacoustic characterization of tinnitus, and no studies have compared two groups with tinnitus that have been recorded in different ways. New investigations using electro-acoustic devices are necessary to standardize psychoacoustic measurements and create and validate new tools for sound therapy to treat it.

By utilizing age clustering to enhance clinical profiling, a separation between young and old patients confirmed some findings from the traditional method for adjusting for age. At the same time, some other significant results were no longer replicated. Within each age cluster, there were no significant differences in the level of education between online participants and outpatients. Differences in presbyacusis and dyslipidemia were also not replicated within clusters of similar age.

After applying the Bonferroni correction, it was found that the differences in acoustic trauma persisted between the young patients from the online cohort and outpatients. However, the differences in hyperacusis and neck pain among the older patients remained insignificant. Additionally, the variables found to be significantly different between online participants and outpatients were ear problems, MD, hyperacusis for the younger group, and severe hyperacusis for the older cluster.

To summarize this first part, self-report questionnaires like ESIT-SQ are beneficial for standardizing clinical profiling in people with chronic tinnitus. However, these instruments should only be used as screening tools, and all medical or psychological conditions, such as anxiety and depression, hearing loss, or MD, should be supported by a medical diagnosis.

Our findings indicate differences in the tinnitus profile between the online and outpatient surveys, including the level of education, hearing disorders related to tinnitus (MD and hyperacusis), and difficulty sleeping. Moreover, there were variations in tinnitus characteristics between the two samples, such as the type of onset, duration, the report of mitigating factors, and the use of treatments. Even after adjusting both groups for age and sex, there were still differences in the tinnitus profile, such as the total duration of tinnitus, duration of debilitating tinnitus, number of perceived sounds, subjective changes in hearing, and factor-increasing tinnitus. Performing the analysis separately within clusters of patients of the same age challenges some of these differences and highlights the role of acoustic trauma in young patients with tinnitus.

5.2 Vestibular Migraine Cohort

In our second study, we aimed to estimate the prevalence of tinnitus and hyperacusis in patients with VM and to determine their association with hearing loss, anxiety, and depression. This is the first report investigating hearing loss, tinnitus, and hyperacusis in patients with VM.

Our cross-sectional study found that the prevalence of tinnitus in patients with VM is 76%, and hyperacusis is 60%. The mean age of the case series is 46.40 ± 13.74, comparable to previous studies on VM[174,175]. The age of onset for VM in our study was similar to that in other reported data, which was 39 ± 13.10 compared to 39.4 ± 8.2[176], but somewhat higher in others, which were 31.8 ± 13.3 or 35.7 ± 14.4, respectively[175,177]. The majority of patients in our series were women (90%, only 3 out of 51 patients were men), which is in contrast with the higher men/women ratio reported in the literature, which is 1:3[175], and in other studies where the prevalence of women is between 70-80%[178,179].

The hearing thresholds in VM patients were mainly normal, with a PTA below 25 dB HL in most cases, even among patients who reported tinnitus (VMt). Only 10% of our patients had hearing loss, which increased to 12% when including patients with tinnitus. However, most patients with tinnitus had normal hearing thresholds. These numbers are lower than those reported by other studies, which found hearing loss rates of 14-44% in individuals[175,178–181]. Nonetheless, other studies support our findings, with rates of 7-15%[182–184]. No previous studies have examined hearing loss in patients with both VM and tinnitus, and high-frequency hearing loss (>8000 Hz) is unlikely to explain tinnitus in these patients, especially high-pitch tinnitus.

Tinnitus and VM are closely associated, with varying prevalence rates reported in the literature. Studies have found that around 38-46% of VM patients may experience tinnitus at some point[185]. However, in our sample, the prevalence rate was higher (76%) than what has been previously reported, which ranges between 20%-50%[175,177–179,181,185]. Nevertheless, none of these studies have explored the psychoacoustic and psychometric features of tinnitus in VM patients. The Tinnitus Handicap Inventory (THI), commonly used to assess tinnitus, has not been used in previous studies to investigate tinnitus in VM patients. In our study, we found that 17% of VM patients (n=35) had a high THI score, ranging between 58-76, and 14% of them had a catastrophic score (THI >78). More extensive studies with a larger sample size are required to understand better the audiological manifestations of VM, including the tinnitus phenotype. In our sample, only one patient experienced pulsatile tinnitus, which has been previously linked to migraine

in some studies[186]. This patient, however, did not respond to migraine medication and was evaluated for other vascular pathologies. Phonophobia and hyperacusis are both associated with migraine and VM, but they are not the same, although they are often reported together in the literature. The prevalence of hyperacusis ranges from 6% to 17% in the general population[187]. In our study, 60% of patients showed hypersensitivity to sound, measured as hyperacusis. Nevertheless, previous studies on VM patients show that only around 30% of patients experience hyperacusis[175,179,183]. Neff's study showed that 80% of patients with VM experience phonophobia[178]. No studies have measured patients' sound sensitivities using validated questionnaires. According to the GÜF findings, 40% of our group has a moderate impairment, and 26% have a severe disability.

Vertigo can be a stressful experience that may trigger fear, phobia, and other psychiatric disorders. Vestibular and mental illnesses have a high comorbidity rate, ranging from 30% to 50%[188]. The prevalence of anxiety in VM sufferers is higher than in patients without vertigo or healthy controls [189]. The HADS is widely used to evaluate emotional distress in nonpsychiatric clinics. Studies indicate that people with vestibular problems had higher HADS scores [174]. Our study presents data from the literature with a mean HADS of 13.28 ± 7.34, anxiety subscales of 8.2 ± 4.78, and depression subscales of 5.37 ± 4.12. Similarly, Kim et al. showed HADS 13.08 ± 5.11, HDASa 7.12 ± 3.29, and HADSd 5.96 ± 3.33 [174]. Another study was not separated by subscale, but the mean HADS score was 14[190].

No explicit research has been conducted on residual inhibition (RI) in individuals with tinnitus and VM. Our study discovered that 50% of patients experienced the disappearance of tinnitus after residual inhibition, significantly greater than other studies that found a complete RI in over 34.5% of patients with tinnitus[191]. However, a larger sample size is required to confirm our results, and this therapy could be considered a possible treatment for selected patients.

VM is associated with various otological abnormalities and systemic comorbidities. Aural fullness was the most prevalent audiological symptom in our patient series (33%), particularly associated with tinnitus. The prevalence of this symptom varies in the literature, affecting 14-50% of patients depending on when the condition has been examined[178,179,185]. Cervicalgia (68%) and TMJ (40%) were frequent disorders in our patients. Neck pain has been described as having a lower prevalence of 32% in some literature[192], while TMJ disorders and tinnitus are reported in the literature between 19-40%, similar to our patients[167,193].

The most common systemic illnesses in our VM cohort were hypertension (32%) and thyroid problems (24%). These findings are consistent with previous research[192], although one study reported a lower prevalence of hypertension (12.3%)[176]. Furthermore, another study indicated that the prevalence of thyroid disease among VM patients (5%) resembled that of the general population[176].

According to research by Batts and Stankovic (2024) and Gedik et al. (2024), the development of tinnitus has been linked with noise exposure, with a higher occurrence of the condition in individuals who have experienced noise exposure. In our sample group, noise exposure was identified as a trigger for tinnitus, with 15% of our patients reporting it as a cause of their condition[194,195].

However, it is important to note that our study has some limitations. Firstly, the data collected through psychometric questionnaires is self-reported, which may result in recall bias and affect the accuracy of the information gathered. Additionally, our sample size is relatively small, although our study was designed as a preliminary investigation to highlight the most relevant factors in patients with VM. Further research with larger sample sizes could be conducted to confirm our initial findings.

5.3   Familial Vestibular Migraine Cohort

Genetic predisposition to VM is a complex issue since it overlaps with genetic contribution to migraine itself. While there is evidence to suggest that VM has a genetic component, the available evidence is limited, and the role of inheritance in VM is not fully understood. Therefore, we performed a WES in 4 multi-case VM families for our final objective.

Several studies have reported familial aggregation of VM, suggesting that its prevalence in families was 4 to 10 times higher than in the general population and that the age of onset was lower in patients with a familial history of migraine or vertigo [105]. Thus, first, we described the families' clinical and auditory characteristics, finding great variability between individuals of different families and individuals within the same family. Some individuals experienced migraine with a strong relation to menstruation. The aura was present in several individuals; all of them described it as a visual aura. Vestibular symptoms showed significant duration variability among individuals and the nature of the dizziness experienced. Tinnitus was a common symptom in these patients, and self-reported hyperacusis was present in half of the patients.

We proceeded to identify variants segregating the phenotype through WES and obtained a series of potential genes that could be associated with familial susceptibility to VM. First, we investigated genes already associated with VM. The most significant one is *TRPM7*, which has been identified through WES studies. A nonsense mutation in the *TRPM7* gene has been found in several families with VM, suggesting that alterations in intracellular Ca^{2+} and Mg^{2+} homeostasis by *TRPM7* mutations may contribute to the development of the VM phenotype [121]. Other genetic studies have also reported allelic variants in the *HTR6* and *PRG* genes [122,123]. Although we found a variant in the *TRPM7* gene throughout our families, none passed the filters since they were considered Low or modifier impact and had a low CADD score or a MAF>0.1.

Genes related to FHM and Episodic ataxia were also considered during the filtering and prioritization since it is a rare and autosomal dominant disorder characterized by recurrent episodes of migraine with hemiplegic symptoms.

Several genes have been related to FHM; mutations in the *CACNA1A* gene, which encodes the alpha-1A subunit of the P/Q-type calcium channel, are the most common cause of FHM. Most of these mutations are missense variants and deletions [196], accounting for 50–75% of FHM cases, and are suggested to initiate a mechanism disrupting the function of Cav2.1 channels [197,198]. In contrast to *CACNA1A*, which regulates ion channels, *ATP1A2* encodes the α2 subunits of the sodium-potassium ATPases pump [199]. The genetic influence of the *ATP1A2* gene involves regulating the work of the α2 subunits of the Na+/K+ ATPase ion transport pump that underlies the process of electrochemical activity in the central nervous system, heart, and skeletal cell membranes [200,201]. Mutations in the *SCN1A* gene, encoding the voltage-gated sodium channel NaV1.1, can disrupt the normal functioning of the sodium channel, leading to an imbalance in sodium levels and contributing to migraine pathophysiology [202]. These channels also control the permeability of sodium ions of the GABA interneurons of the central nervous system [203]. The *KCNA1* gene encodes the α subunit of the potassium channel KV1.1, which is involved in the regulation of neuronal excitability and the repolarization of axons.

Missense mutations of *KCNA1* were first identified in association with the autosomal dominant disorder episodic ataxia type 1 (EA1) [204]. Mutations in the *KCNA1* gene can lead to alterations in the function of the KV1.1 channel, which is involved in the regulation of neuronal excitability and the repolarization of axons, resulting in changes in the excitability of neurons and the development of migraine symptoms [205]. Mutations in the *KCNA1* gene have also been found in FHM.

We found several variants in those genes among all four families, but once again, none of them met the filtering thresholds. We also filtered out genes with low expression in brain and inner ear tissues or genes strongly related to known specific diseases selecting the strongest potential candidate genes for each family.

We found 47 potential candidate genes in family 1 that have passed all filters and are expressed in the brain or inner ear. These genes are involved in various cellular processes, such as protein-protein interactions, gene expression, protein synthesis, and protein degradation.

Several of these genes could interact through their roles in broader biological processes, such as immune responses (*CD247, FERMT1, MAP3K6*), cell adhesion (*ITGA6, ITGB4, TJP1*), and apoptosis (*AIFM2, MOAP1, UNC5B*).

Increased levels of pro-inflammatory cytokines like TNF-α, IL-1β, IL-6, and IL-12 have been found in patients with migraine. Moreover, decreased levels of anti-inflammatory cytokine IL-10, increased CD4+ T cells, and decreased CD8+ T cells with reduced regulatory T cells (CD4+CD25+) have also been observed [206].

Studies have shown that immune cell activity is also altered in VM patients, leading to an imbalance in the immune response and contributing to the development of vestibular symptoms. A study found that VM patients had a distinct proinflammatory signature characterized by elevated levels of cytokines such as IL-1β, CXCL10, and CCL3. This signature was different from that of MD patients and healthy controls [207].

MAP3K6 is part of the mitogen-activated protein kinase (MAPK) pathway and is involved in signaling cascades that regulate inflammation, cell growth, and apoptosis. It plays an essential role in regulating inflammatory responses through its involvement in the JNK and p38 MAPK signaling pathways [208]. Upon oxidative stress or other stimuli, active phosphorylated MAP3K6 activates the downstream JNK and p38 cascades, producing pro-inflammatory cytokines and promoting apoptosis. Its interaction with *MAP3K5* and the balance between their opposing roles is tightly regulated to control inflammation and apoptosis in various pathological contexts [209].

CD247 encodes the CD3 zeta chain and is the gamma subunit of the T cell receptor complex. It plays a crucial role in T cell activation and signaling. *CD247* plays a critical role as the "gatekeeper" of T-cell activation, and its downregulation is associated with suppressed T-cell activation during chronic inflammation [210,211]. The expression levels of *CD247* correlate with T-cell activation status, making it a potential biomarker for

assessing immune function and disease severity in conditions involving dysregulated T-cell responses [212,213].

FERMT1, also known as fermitin family homolog 1 or kindlin-1, is a gene that encodes a protein involved in integrin activation and cell-extracellular matrix adhesion. Evidence suggests that *FERMT1* appears to regulate inflammatory signaling pathways like NF-κB and *NLRP3*, mediating epithelial-mesenchymal transition (EMT) through binding to *NLRP3*, which can influence chronic immune responses such as fibrosis [214,215].

Furthermore, the potential role of cell adhesion in VM could involve complex interactions between cell adhesion molecules (CAM), the Blood Brain Barrier (BBB), neuroinflammation, synaptic function, and vestibular system maintenance. Disruptions in these processes may contribute to the pathophysiology of VM. Thus, mutations in genes related to cell adhesion, like *ITGA6*, *ITGB4*, and *TJP1*, could play a role in VM susceptibility.

Integrins *ITGA6* (integrin alpha-6), *ITGB4* (integrin beta-4), and *ITGB6* (integrin beta-4), along with tight junction protein 1 (*TJP1*, also known as *ZO-1*), are crucial for cell adhesion and maintaining the integrity of the BBB. *ITGA6* and *ITGB4* form a heterodimer known as α6β4 integrin, which is essential for the structural integrity of epithelial cells and signal transduction. This integrin is involved in BBB maintenance, and its disruption can increase BBB permeability, allowing inflammatory mediators to enter the CNS and potentially trigger migraine mechanisms. Moreover, altered integrin signaling could lead to the activation of microglia, resulting in the release of pro-inflammatory cytokines and subsequent neuroinflammation [216,217]. Consequently, elevated levels of inflammatory cytokines in individuals with migraine could exacerbate BBB disruption and contribute to VM pathogenesis.

TJP1 plays a pivotal role in forming and maintaining tight junctions within the BBB. Disruption or altered expression of *TJP1* can result in increased BBB permeability, facilitating the entry of migraine triggers and inflammatory mediators into the CNS and allowing neuroinflammatory processes to affect the brain [218,219]. Furthermore, inflammatory cytokines can downregulate *TJP1* expression or disrupt its function, further compromising BBB integrity and potentially initiating migraine mechanisms. Such inflammation may possibly impact vestibular nuclei and pathways, contributing to VM symptoms.

In summary, no candidate gene can be prioritized in this short list according to the variants found in family 1.

Family two, which had the largest number of sequenced individuals, had the list of candidate genes narrowed down to two: *ZNF469* and *MEGF8*. The CADD > 20 filter could not be applied for this family as the variants did not meet this condition.

The relationship of these genes to VM is not yet established. *ZNF469* encodes zinc-finger protein 469, which is suggested to function as a transcription factor or extra-nuclear regulatory factor involved in the synthesis or organization of collagen fibers. Mutations in *ZNF469* are primarily associated with Brittle Cornea Syndrome (BCS), specifically type 1 and with the transcriptional regulation. Its mutation has also been related to Fanconi anemia [220] and keratoconus [221,222].

BCS is a rare hereditary connective tissue disease characterized by severe ocular manifestations resulting from extreme corneal thinning and fragility, which can lead to irreversible blindness. Additional manifestations include hearing loss, hip dysplasia, and soft skin with abnormal scarring. BCS is classified into two types: type 1, which is caused by mutations in the *ZNF469* gene and it is inherited in an autosomal recessive manner[223,224].

The current literature does not know of a direct relationship between migraines or VM. However, since this gene is involved in the development and maintenance of tissues, it could indirectly influence inflammatory processes or structural aspects of connective tissue that might potentially be related to pain or dizziness.

MEGF8 (Multiple EGF Like Domains 8) encodes a single-pass type I membrane protein of unknown function that contains several EGF-line domains, Kelch repeats, and PSI domains. It is involved in the development of the nervous system and other structures during embryonic development. This gene is also related to Ca^{2+} binding and signaling receptor activity. Mutations in *MEGF8* are associated with Carpenter Syndrome Type 1 and type 2 [225].

Carpenter Syndrome is a rare autosomal disorder presenting a complex array of symptoms characterized by craniosynostosis. This condition affects brain development and can result in increased intracranial pressure, craniofacial asymmetry, and distinctive facial features such as a flat nasal bridge, down-slanting palpebral fissures, low-set and abnormally shaped ears, underdeveloped jaws, and abnormal eye shape. Dental abnormalities, including small primary teeth and vision problems, are also common [226].

Due to its involvement in the development of the nervous system, it could be relevant in neurological disorders, although there is no known direct relationship with migraines in the literature.

In summary, no candidate gene can be prioritized in this short list according to the variants found in family 2, and other types of variants, such as structural variants or non-coding variants, may explain the phenotype.

In the analysis of family three, 11 variants have been identified in 11 candidate genes. Some of these genes are particularly relevant because they participate in direct processes of pain regulation. For instance, *GABBR1*, which encodes subunit 1 of the gamma-aminobutyric acid (GABA) type B receptor, is involved in the modulation and processing of pain. It is widely expressed at the brain level, mainly in the cerebral hemispheres, cerebellum, nucleus accumbens and putamen, hypothalamus, and frontal cortex. GABA is the main inhibitory neurotransmitter of the central nervous system in mammals. *GABBR1* functions as a heterodimer with *GABBR2* [227,228]. Within the GABA receptor, *GABBR1* is the one that has the role of binding agonists directly to the GABA receptor [229]. This binding causes a conformational change that triggers a signal through guanine nucleotide-binding proteins (G proteins) and modulates the activity of downstream effectors such as adenylate cyclase [230]. Defects in this gene may underlie brain disorders, mainly associated with schizophrenia and epilepsy [231,232].

We also found expression of this gene in spiral ganglion neurons, primarily in type 2 neurons and to a lesser extent in type 1C neurons and Schwann cells, as indicated by the gEAR database. *GABBR1* also shows expression in the trigeminal nucleus, a crucial region in transmitting facial pain signals. This suggests that *GABBR1* could be involved in modulating pain related to VM.

The involvement of *GABBR1* in pain modulation has been related to migraine susceptibility and vestibular symptoms. Many studies have implicated GABA receptors in the study of migraine as well as in its modulation and its usefulness as a possible therapeutic target. [233]. Thus, this alteration of neuronal excitability and involvement in pain signaling processes may be implicated in the pathophysiology of VM.

POSTN is another significant candidate gene, encoding an extracellular matrix protein integral to tissue development and regeneration, including wound healing. The protein it encodes, Periostin, binds to integrins, thereby promoting cell adhesion and migration [234]. Although alterations in this gene are associated in the literature with myocardial infarction and melorheostosis, as well as with metabolic pathways involved in the amplification and expansion of cancerous and metastatic processes, its role in inflammatory processes makes it pertinent to research on VM and related symptoms.

Therefore, considering its clear involvement in relevant pain modulation and signaling mechanisms, GABBR1 can be considered a candidate gene for the VM phenotype in this family.

Family four had the highest number of genes at the end of the filtering process, resulting in 84 variants. Among these genes, several were selected for their notable involvement in critical biological processes: *ANK3* for cell motility, *IL17RD* and *NRG2* for growth factors and interleukins, *SPTBN2* and *DNAJB12* for signaling pathways, and *ATP10B* and *SPG11* for membrane stability.

ANK3 (Ankyrin 3, also known as Ankyrin-G) is a particularly relevant gene because this gene has two mutated variants. Ankyrins are a family of proteins that function to anchor integral membrane proteins to the cytoskeleton, playing a crucial role in cell motility and membrane maintenance. Ankyrin 3, specifically, functions in the axonal initial segment and the nodes of Ranvier in neurons of the central and peripheral nervous system. Its expression is predominantly found in the brain, particularly in the cerebellum. The main diseases associated with the *ANK3* mutation are intellectual development disorders and medulloblastoma, as well as alterations in the development of the nervous system. [235]. Psychiatric disorders, such as bipolar disorder and depression, have been associated with certain variants of *ANK3*, suggesting that this gene may influence susceptibility to these conditions. [236–238]

Although no direct relationship has been established between *ANK3* and VM, its role in neuronal membrane stability and signaling may indirectly influence migraine susceptibility. Furthermore, its extensive expression in the cerebellum could be involved in the development of vestibular symptoms, such as instability.

On the other hand, *IL17RD* (Interleukin 17 Receptor D), also known as *SEF*, encodes a membrane protein that belongs to the interleukin-17 receptor (IL-17R) protein family. This protein influences fibroblast growth factor signaling and can either inhibit or stimulate growth mediated by MAPK/ERK signaling pathways. [239,240]. It has homogeneous expression across different brain areas, with a higher concentration in the hypothalamus and hippocampus. Although there is no direct relationship with VM, *IL17RD* encodes a protein involved in signaling and inflammatory mechanisms. Consequently, it could play an indirect role in modulating the inflammatory state, altering pain perception, and producing some symptoms related to VM

The most relevant disease associated with mutations in this gene is Kallmann Syndrome, a genetic defect associated with congenital hypogonadotropic hypogonadism and anosmia or hyposmia and arteriovenous malformations of the brain [241].

NRG2 (Neuregulin 2) encodes a member of the neuregulin family, which functions as growth and differentiation factors through their interaction with ERBB receptors. This protein primarily induces the growth and differentiation of epithelial, neuronal, and glial cells. Its expression is particularly high in the cerebellum. This gene is located near the locus associated with Charcot-Marie-Tooth demyelinating disease (CMT), suggesting its mutation may be related to CMT without being the direct cause. CMT is a hereditary motor and sensory neuropathy that affects the peripheral nervous system [242]. *NRG2*, considering its role in neuronal differentiation, may be indirectly related to some common symptoms among patients with VM, as it can induce neurons to an altered state of excitability. Additionally, the clinical manifestations of muscle weakness observed in neuropathies could be associated with the spectrum of instability symptoms experienced by these patients. Altered somatosensory and proprioceptive activity could generate a state of increased susceptibility to instability.

SPTBN2 is one of the genes involved in signaling pathways, and it is associated with degenerative alterations at the brain level.

SPTBN2 (Spectrin Beta, Non-Erythrocytic 2) encodes the beta subunits of spectrin, which are essential components of the cell membrane cytoskeleton. *SPTBN2* plays a crucial role in regulating the glutamate signaling pathway by stabilizing the glutamate transporter EAAT4 at the plasma membrane surface. Its highest expression is found in brain tissue, particularly in the cerebellum.

Mutations in this gene are associated with spinocerebellar ataxia 5 (SCA5) and autosomal recessive spinocerebral ataxia 14 (SCAR14), as well as with alterations in MAPK family signaling cascades [243–245]. SCA5 is an autosomal dominant cerebellar ataxia characterized by the early onset of cerebellar signs, including eye movement abnormalities due to cerebellar degeneration, and progressive incoordination [246,247]. SCAR14 is a neurological disorder characterized by psychomotor developmental delays, severe ataxia, eye movement abnormalities, intellectual disability, and cerebellar atrophy[248,249].

Considering the cerebellum's crucial role in postural control and the direct relationship between ataxia and vestibular symptoms, particularly those related to instability and the sensation of movement, *SPTBN2* may have a direct connection to the symptomatological origin of VM.

SPTBN2 has also been linked to episodic ataxia type 6. Although mutated variants of another episodic ataxia gene, *CACNA1A*,[250] have previously been identified in the family, these variants were discarded due to their failure to pass the applied filters and their low pathogenicity or high prevalence in the study population.

ATP10B (ATPase Phospholipid Transporting 10B) facilitates glycosylamide flippase activity and phosphatidylcholine lipase activity, playing a key role in the organization of the lysosomal membrane in cortical neurons. It is also a component of the phospholipid translocation ATPase complex. ATP10B is expressed in the spinal cord and throughout the cochlea, particularly in the outer hair cells. Mutations in this gene are associated with deafness, autosomal dominant 33 (DFNA33)[251] and Waardenburg Syndorme, Type 4C (WS4C) [252]. As mentioned above, the activity exerted by this protein on the membrane of neurons could induce states of hyperexcitability or alterations in its components, potentially contributing to the migraines experienced by patients with VM.

Finally, SPG11 (Vesicle Trafficking Associated, Spatacsin) encodes a transmembrane protein that is phosphorylated in response to DNA damage. Mutations in this gene are a known cause of spastic paraplegia type 11 and CMT disease type 2X. SPG11 is expressed uniformly throughout the brain and spinal cord. Alterations in this gene lead to symptoms such as muscle weakness, dysarthria, and peripheral neuropathy. Similar to NRG2, these symptoms can overlap with the spectrum of vestibular symptoms observed in patients with VM [253,254].

In summary, *ANK3* and *SPTBN2* have emerged as the most powerful genes in this family and can be postulated as candidate genes for familial VM. The extensive expression of *ANK3* in the brain, coupled with its involvement in signaling mechanisms that may alter pain perception and influence migraine susceptibility, positions it as a strong candidate gene for VM. *SPTBN2* is directly related to balance disorders due to its wide expression in the cerebellum and its association with spinocerebellar disorders. This gene may also be linked to the broad spectrum of symptoms characteristic of VM, particularly those related to dizziness. Additionally, *SPTBN2* is associated with visual disturbances, which can result from altered eye movements, alterations that sometimes manifest themselves during vestibular migraine attacks in patients with VM.

6 CONCLUSIONS

1. Self-reported tinnitus cannot accurately identify tinnitus characteristics, and clinical and audiological assessments are essential to establish a precise diagnosis, and to define clinical subgroups.
2. Age-based cluster analysis could improve clinical profiling using the ESIT-SQ among different subgroups of patients with tinnitus.
3. Tinnitus and hyperacusis are very common symptoms in patients with VM, regardless of hearing thresholds.
4. Hyperacusis is associated with tinnitus, anxiety, and depression in VM patients.
5. The observed higher prevalence of vestibular migraine among first-degree relatives strongly indicates a genetic component. This study has identified a genetic linkage associated with specific missense variants, highlighting the potential genetic factors influencing this condition.
6. The genes involved in familial VM are related to pain perception mechanisms, the maintenance and structure of the cell membrane, and inflammatory processes.

6 CONCLUSIONES

1. Las autoevaluaciones de acúfenos no identifican con precisión sus características; son esenciales las evaluaciones clínicas y audiológicas para un diagnóstico preciso y definir subgrupos clínicos.
2. El análisis de clúster por edad podría mejorar el perfil clínico utilizando el ESIT-SQ entre diferentes subgrupos de pacientes con acúfenos.
3. Los acúfenos y la hiperacusia son síntomas muy comunes en pacientes con MV, independientemente de los umbrales auditivos.
4. La hiperacusia está asociada con acúfenos, ansiedad y depresión en pacientes con MV.
5. La mayor prevalencia observada de migraña vestibular entre familiares de primer grado indica claramente un componente genético. Este estudio ha identificado una relación genética asociada a variantes missense específicas, lo que pone de relieve los posibles factores genéticos que influyen en esta afección.
6. Los genes involucrados en la MV familiar están relacionados con mecanismos de percepción del dolor, el mantenimiento y estructura de la membrana celular y con los procesos inflamatorios.

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Cuestionario de cribado de la Escuela Europea de Investigación Interdisciplinaria en Acúfenos (ESIT-SQ)

Este cuestionario consta de dos partes.

En la parte A, le pedimos ciertas características generales y personales como la edad, la estatura, preguntas sobre el estilo de vida y condiciones que pueda padecer. Todas las personas pueden completar la parte A, incluso si usted nunca ha presentado acúfenos. El tiempo estimado para completar esta parte es de 5 minutos.

Si usted ha presentado acúfenos durante el pasado año, se le harán más preguntas relacionadas con los acúfenos en la parte B. El tiempo estimado para completar la parte B es de entre 5 y 10 minutos, dependiendo de sus respuestas.

PARTE A. DATOS PERSONALES

Para las siguientes preguntas, dé la respuesta que mejor se ajuste a usted o a su experiencia. Puede elegir más de una opción.

A1	Edad (años)
	_ _

A2	Sexo, al nacer:
	☐ Hombre ☐ Mujer ☐ Intersexual ☐ Prefiero no contestar

A3	¿Cuál es su estatura?
	_ _ _ cm

A4	¿Cuál es su peso?
	_ _ _ kg

A5	¿Cuál es la titulación académica más alta que ha alcanzado?
	☐ No escolarización
	☐ Educación Primaria
	☐ Educación Secundaria
	☐ Bachillerato (Pre-universitaria)
	☐ Estudios universitarios

A6	¿Cuál es la cantidad media de bebidas alcohólicas que consume a la semana? Una bebida equivale a 125ml de vino, 330ml de cerveza o 40 ml de bebidas destiladas
	_ _

A7	¿Cuál de las siguientes opciones describe mejor su consumo de tabaco?
	☐ Nunca he sido fumador ☐ Fumador ☐ Ex fumador

A8	¿Cuántos familiares de primer grado (padres, hijos, hermanos) conoce que tengan acúfenos o pérdida de audición? Por favor, escriba un numero en cada uno de los familiares.
	__ Padre __ Madre
	__ Hermanos __ Hermanas
	__ Hijos __ Hijas

A9	¿Sufre usted vértigo (sensación de giro o inclinación)?

☐ Nunca

☐ Sí, menos de un episodio al año

☐ Sí, al menos un episodio al año

A10	¿Ha sido diagnosticado de algún problema de oído?

Puede elegir más de una opción.

☐ Trauma acústico (inducido por ruido)

☐ Barotrauma ótico (causado por cambios de presión bruscos)

☐ Presbiacusia (pérdida de audición por envejecimiento)

☐ Hipoacusia súbita

☐ Otras pérdidas de audición

☐ Enfermedad de Ménière

☐ Neurinoma del acústico (tumor del nervio auditivo)

☐ Otitis aguda (inflamación del oído)

☐ Otitis serosa o disfunción de la trompa de Eustaquio

☐ Otitis crónica (p. ej. perforación timpánica, colesteatoma)

☐ Otosclerosis (fijación de la cadena de huesecillos)

☐ Otros problemas de oído. Por favor, especifique _____

☐ No

A11	¿Se ha sometido alguna vez a alguno de los siguientes procedimientos?

Puede elegir más de una opción.

☐ Cirugía de oído

☐ Cirugía dental

☐ Neurocirugía

☐ Punción lumbar

☐ Quimioterapia

☐ Radioterapia de cabeza y cuello

☐ Terapia electroconvulsiva

☐ Otro procedimiento. Por favor, especifique _____

☐ Ninguna de ellas

A12	Durante la semana pasada, ¿le han supuesto un problema los sonidos externos, siendo muy fuertes o molestos para usted cuando resultaban normales para otras personas a su alrededor? Nota: los sonidos externos hacen referencia a cualquier sonido que no sea su acúfeno, p. ej. sonidos ambientales, habla, música.

☐ No, no son un problema

☐ Sí, un problema leve

☐ Sí, un problema moderado

☐ Sí, un problema grave

☐ Sí, un problema muy grave

A13	¿Tiene algún otro problema de audición, como comprender palabras en ambientes ruidosos?

☐ Sí, no puedo escuchar nada en absoluto

☐ Sí, gran dificultad

☐ Sí, moderada dificultad

☐ Sí, leve dificultad

☐ Ninguna dificultad

☐ No sabe/no contesta

A14	¿Utiliza alguno de los siguientes dispositivos?
	Puede elegir más de una opción.

☐ Audífono
☐ Implante coclear
☐ Generador de sonidos
☐ Dispositivos combinados (audífono y generador de ruido en el mismo dispositivo)
☐ Ninguno

A15	¿Padece alguno de los siguientes problemas de dolor?
	Puede elegir más de una opción.

☐ Dolor de cabeza
☐ Dolor cervical
☐ Dolor de oídos
☐ Dolor de la articulación temporomandibular
☐ Dolor facial
☐ Otros. Por favor, especifique _____
☐ No

A16	¿Ha sido diagnosticado por algún medico de alguna de las siguientes patologías? .
	Puede elegir más de una opción.

Vía oral:
☐ Trastornos de la articulación temporomandibular
☐ Problemas dentales
Neurológico:
☐ Meningitis
☐ Esclerosis múltiple
☐ Epilepsia
☐ Ictus
☐ Otra enfermedad cerebrovascular
☐ Demencia
☐ Otra enfermedad neurológica
Psiquiátrico o psicológico:
☐ Ansiedad
☐ Depresión
☐ Trauma emocional
☐ Estrés excesivo
Trastornos del sueño:
☐ Dificultad para conciliar el sueño
☐ Dificultad para mantenerse despierto
Cardiovascular:
☐ Presión sanguínea alta
☐ Presión sanguínea baja
☐ Infarto de miocardio (ataque al corazón)
Endocrino y metabólico:
☐ Trastorno tiroideo
☐ Diabetes
☐ Hiperinsulinismo
☐ Colesterol alto
Reumatológico y autoinmune:
☐ Artritis reumatoide

☐ Lupus eritematoso sistémico
Otorrinolaringológico:
☐ Sinusitis crónica
☐ Desviación del tabique nasal
Infecciosos:
☐ Sífilis
☐ VIH
☐ Enfermedad de Lyme
Otros:
☐ Anemia
☐ Inestabilidad u otro desorden del equilibrio
☐ Acidez/Reflujo gastroesofágico
☐ Bolo histérico
☐ Otro. Por favor especifique _____
☐ Ninguno

A17	Los acúfenos consisten en la percepción de ruido en su cabeza o en sus oídos (como un pitido o un zumbido) que no proceden de ninguna fuente externa a su cabeza. Durante el último año, ¿ha sentido acúfenos en su cabeza, en ambos oídos o en alguno de ellos, que hayan durado más de 5 minutos seguidos?

☐ Sí, la mayor parte del tiempo
☐ Sí, gran parte del tiempo
☐ Sí, algunas veces
☐ No, en el último año
☐ No, nunca
☐ No sabe/no contesta

Gracias por completar la sección A. Si ha contestado "Sí" en la pregunta A17, por favor continúe con la sección B. Si ha contestado "No" o "No sabe/no contesta" en la pregunta A17, ha finalizado el cuestionario. Gracias por participar en esta encuesta.

PARTE B. CARACTERISTICAS DEL ACUFENO

Gracias por completar la parte A. Para las siguientes preguntas, por favor responda de la manera que mejor describa su acúfeno y su relación con otras enfermedades. En algunas preguntas puede seleccionar más de una opción.

B1	¿Con qué frecuencia de media presenta acúfenos?

☐ A diario o casi a diario
☐ Una vez por semana aproximadamente
☐ Una vez al mes aproximadamente
☐ Cada pocos meses
☐ Una vez al año

B2	¿Cómo describiría su acufeno durante el día?

☐ Constante: siempre o casi siempre puede oírlo en una habitación silenciosa
☐ Intermitente: "viene y va", no siempre puede oírlo en una habitación silenciosa

B3	¿Cuánto tiempo hace que apareció su acúfeno?

_ _ meses
_ _ años

☐ No lo sé

B4	Durante el último año, ¿Cuánto le preocupa, molesta o trastorna su acúfeno, cuando éste empeora?

☐ Mucho
☐ Moderadamente
☐ Ligeramente
☐ No me preocupa
☐ No lo sé

Si ha contestado "No me preocupa" o "No sabe/no contesta", por favor pase a la pregunta B6.

B5	¿Hace cuánto tiempo comenzó el acúfeno a molestarle?

_ _ meses
_ _ años

☐ No lo sé

B6	Aunque, la mayoría de pacientes presentan acúfenos de un solo tipo, algunos pueden escuchar distintos sonidos. ¿Usted escucha uno o varios sonidos diferentes?

☐ Un sonido ☐ Más de un sonido diferente

En caso de que escuche más de un sonido distinto, por favor intente contestar lo que mejor se ajuste a su acúfeno más molesto, de entre las siguientes preguntas.

B7	¿Cómo comenzó su acúfeno?

☐ De forma gradual ☐ Brusco ☐ No sabe

B8	Si usted ha reflejado algún proceso/condición en la pregunta A9, A10, A11, A12, A13, A15 o A16, por favor enumérelas a continuación, y escriba al lado de cada una de ellas si sucedieron ANTES, DESPUÉS o más o menos AL MISMO TIEMPO que el inicio del acúfeno.

_____
_____
_____
_____

B9	¿Estuvo relacionado el comienzo de su acúfeno con (puede elegir más de una opción):

☐ Exposición a sonidos fuertes
☐ Cambio en la audición
☐ Exposición al cambio de presión atmosférica (p. ej. vuelo o inmersión)
☐ Fiebre, resfriado común o alguna otra infección
☐ Sensación de taponamiento o presión en los oídos
☐ Estrés
☐ Traumatismo craneal
☐ Traumatismo cervical (p. ej. latigazo cervical)
☐ Otro. Por favor, especifique _____
☐ Ninguno

B10	¿Estaba usted en tratamiento con alguno de los siguientes medicamentos cuando comenzó el acúfeno?
	Puede elegir más de una opción.

☐ Aspirina
☐ Analgésicos. Por favor, especifique _____
☐ Corticoides orales. Por favor, especifique _____
☐ Antibióticos. Por favor, especifique _____
☐ Antidepresivos. Por favor, especifique _____
☐ Quinina (calambres musculares, malaria)

☐ Diuréticos. Por favor, especifique _____

☐ Otros medicamentos. Por favor, especifique _____

☐ No

☐ No losé

B11	¿Cree que alguno de los problemas mencionados anteriormente o algún otro está relacionado con la aparición de su acúfeno?

Puede elegir hasta 3 respuestas - por favor elija las más importantes.

☐ No

☐ Sí. Por favor, especifique _____

B12	El volumen de su acúfeno, ¿es estable o fluctúa a lo largo del día?

☐ Estable

☐ A veces fluctúa

☐ Siempre fluctúa

☐ No sabe/no contesta

B13	¿Cómo suena su acúfeno?

☐ Tono puro

☐ Ruido

☐ Musical

☐ Como los grillos

☐ Otros. Por favor, especifique _____

B14	Por favor, describa la frecuencia de su acúfeno:

☐ Frecuencia alta

☐ Frecuencia media

☐ Frecuencia baja

☐ No sabe

B15	¿Dónde percibe su acúfeno?

☐ Oído derecho

☐ Oído izquierdo

☐ Ambos oídos, peor en el derecho

☐ Ambos oídos, peor en el izquierdo

☐ Igual en ambos oídos

☐ Dentro de la cabeza

☐ Otro. Por favor, especifique _____

☐ No sabe/no contesta

B16	¿Es su acúfeno rítmico?

☐ No

☐ Sí, siguiendo el latido del corazón (se puede comprobar sintiendo el pulso al mismo tiempo que escucho el acúfeno)

☐ Sí, siguiendo la respiración

☐ Sí, siguiendo los molos movimientos de la cabeza, el cuello, la mandíbula o los músculos de la cabeza

☐ Otro. Por favor, especifique _____

B17	¿Alguna vez ha escuchado su acúfeno un médico?

☐ Sí ☐ No

B18	Su acúfeno se reduce por (puede elegir	B19	Su acúfeno se ve incrementado por

más de una opción):

- ☐ Ambientes muy silenciosos
- ☐ Sonidos de baja intensidad
- ☐ Sonidos de alta intensidad
- ☐ Movimientos de cabeza
- ☐ Apretar los dientes o mover la mandíbula
- ☐ Presionar la cabeza, el cuello, u otra área alrededor de los oídos
- ☐ Echar una siesta
- ☐ Sueño de buena calidad
- ☐ Conducir
- ☐ Estrés o ansiedad
- ☐ Estar relajado
- ☐ Consumo de alcohol
- ☐ Consumo de café
- ☐ Medicación
- ☐ Uso de audífonos
- ☐ Otros. Por favor especifique _____ _____
- ☐ Ninguna

(puede seleccionar más de una opción):

- ☐ Ambientes muy silenciosos
- ☐ Sonidos de baja intensidad
- ☐ Sonidos de alta intensidad
- ☐ Movimientos de cabeza
- ☐ Apretar los dientes o mover la mandíbula
- ☐ Presionar la cabeza, el cuello, u otra área alrededor de los oídos
- ☐ Echar una siesta
- ☐ Mala calidad del sueño
- ☐ Conducir
- ☐ Estrés o ansiedad
- ☐ Estar relajado
- ☐ Consumo de alcohol
- ☐ Consumo de café
- ☐ Medicación
- ☐ Uso de audífonos
- ☐ Otros. Por favor especifique _____ _____
- ☐ Ninguna

B20	Durante el último año, ¿ha visitado a su médico de familia o a un profesional sanitario en una clínica u hospital debido a su acúfeno?

- ☐ Sí, 5 o más visitas
- ☐ Sí, de 2 a 4 visitas
- ☐ Sí, solo una vez
- ☐ No
- ☐ No sabe

B21	Actualmente, ¿Está recibiendo alguna de los siguientes tratamientos para el manejo de los acúfenos? Puede elegir más de una opción.

- ☐ Tratamiento psiquiátrico
- ☐ Tratamiento psicológico
- ☐ Tratamiento audiológico
- ☐ Fisioterapia
- ☐ Autotratamiento (p. ej. suplementos dietéticos, grupos de ayuda, relajación
- ☐ Otro. Por favor, especifique _____
- ☐ Ningún tratamiento

B22	¿Piensa que alguna de los factores mencionados previamente o algún otro están relacionadas con periodos de aumento de los acúfenos? Puede elegir hasta 3 respuestas - por favor elija las más importantes.

- ☐ No
- ☐ Sí. Por favor, especifique _____

Gracias por participar en esta encuesta.

Scuola Europea per la Ricerca Interdisciplinare sull'Acufene – Questionario Di Monitoraggio (ESIT-SQ)

Il questionario è suddiviso in due sezioni.

Nella sezione A, poniamo domande relative ad alcune caratteristiche personali, come età, altezza, stili di vita ed eventuali patologie. Chiunque può completare la sezione A, anche chi non avesse mai sofferto di acufene. Il tempo stimato per completare questa sezione è di 5 minuti.

Nel caso lei abbia sofferto di acufene nell'ultimo anno, le saranno poste alcune ulteriori domande relative all'acufene, sezione B. Il tempo totale stimato per completare la sezione B è compreso tra i 5 e i 10 minuti, a seconda delle sue risposte.

SEZIONE A. CARATTERISTICHE INDIVIDUALI

La preghiamo di indicare per ciascuna delle domande seguenti, la risposta che meglio descrive lei e le sue esperienze. Per alcune domande è possibile selezionare più di una opzione.

A1	Età (in anni)
	_ _
A2	Sesso (alla nascita):
	☐ Maschio ☐ Femmina ☐ Intersessuale ☐ Preferisce non rispondere
A3	Quanto è alto?
	_ _ _ cm
A4	Quanto pesa?
	_ _ _ kg
A5	Qual è il suo titolo di studio?
	☐ Nessun titolo di studio
	☐ Licenza elementare
	☐ Licenza media inferiore
	☐ Licenza di media superiore
	☐ Laurea o specializzazione post-laurea
A6	Quante bevande alcoliche consuma, in media, in una settimana? Una bevanda equivale un bicchiere di vino da 125 ml, 330 ml di birra o 40 ml di superalcolici
	_ _
A7	Quale delle seguenti opzioni descrive meglio la sua abitudine al fumo?
	☐ Non ho mai fumato ☐ Sono un fumatore ☐ Sono un ex-fumatore
A8	Quanti, tra i suoi parenti di primo grado (genitori, figli o fratelli/sorelle) soffrono di acufene o di perdita dell'udito? Precisare il numero accanto ad ogni categoria.
	__ Padre __ Madre
	__ Fratelli __ Sorelle
	__ Figli __ Figlie

A9 Ha mai sofferto di vertigini (sensazione di capogiro o perdita di equilibrio)?
- ☐ Mai
- ☐ Si, meno di una volta all'anno
- ☐ Si, almeno una volta all'anno

A10 Le è mai stata diagnosticata un'altra patologia del sistema uditivo?
E' possibile selezionare più di una opzione.
- ☐ Trauma acustico (causato da rumori forti)
- ☐ Barotrauma dell'orecchio (causato da improvvisi sbalzi di pressione atmosferica)
- ☐ Presbiacusia (invecchiamento dell'orecchio)
- ☐ Improvvisa perdita dell'udito
- ☐ Altra perdita dell'udito
- ☐ Sindrome di Meniere
- ☐ Neuroma acustico (tumore al nervo acustico)
- ☐ Otite acuta (infiammazione dell'orecchio)
- ☐ Otite sierosa o disfunzione della tromba di Eustachio
- ☐ Otite cronica (perforazione del timpano, colesteatoma)
- ☐ Otosclerosi (ridotta mobilità degli ossicini uditivi)
- ☐ Altre patologie uditive (specificare quali) _____
- ☐ Nessuna patologia

A11 Hai mai subito uno o più dei seguenti trattamenti medici?
E' possibile selezionare più di una opzione.
- ☐ Chirurgia dell'orecchio
- ☐ Chirurgia odontoiatrica
- ☐ Neurochirurgia
- ☐ Rachicentesi (puntura lombare)
- ☐ Chemioterapia
- ☐ Radioterapia al collo o alla testa
- ☐ Terapia elettroconvulsiva
- ☐ Altro trattamento (specificare quale) _____
- ☐ Nessuno dei trattamenti indicati sopra

A12 Nel corso dell'ultima settimana, le sembra che i rumori esterni abbiano rappresentato un problema, nel senso che li ha percepiti come forti o fastidiosi, mentre gli stessi suoni apparivano normali alle persone intorno a lei?
Nota: per rumori esterni ci riferiamo a suoni non legati all'acufene, per esempio, rumori provenienti dall'ambiente, brusìi o musica
- ☐ No, i rumori non sono stati un problema
- ☐ Si, hanno rappresentato un problema di modesta entità
- ☐ Si, hanno rappresentato un problema di seria entità
- ☐ Si, hanno rappresentato un problema di entità molto seria
- ☐ Sì, hanno rappresentato un fortissimo disagio per me

A13 Ha attualmente qualche altro disturbo di udito, come ad esempio difficoltà ad ascoltare chi parla in un ambiente rumoroso?
- ☐ Sì, non sento niente
- ☐ Sì, una notevole difficoltà
- ☐ Sì, una media difficoltà
- ☐ Sì, una lieve difficoltà
- ☐ Nessuna difficoltà

☐ Non so

A14	Fa uso di uno o più dei seguenti dispositivi?

E' possibile selezionare più di una opzione.

☐ Apparecchio acustico

☐ Impianto cocleare

☐ Generatore di suoni

☐ Combinazione di dispositivi (apparecchio acustico e generatore di suono nello stesso apparecchio)

☐ Nessun dispositivo

A15	Soffre di una o più delle seguenti sindromi dolorose?

E' possibile selezionare più di una opzione.

☐ Mal di testa

☐ Dolore al collo

☐ Dolore all'orecchio

☐ Dolore alle articolazioni temporo-mandibolari

☐ Dolore al volto

☐ Altro, specificare _____

☐ Nessuna

A16	Le sono mai state diagnosticate una o più delle seguenti patologie?

E' possibile selezionare più di una opzione.

Patologie orali:

☐ Disfunzione delle articolazioni temporo-mandibolari

☐ Problemi odontoiatrici

Patologie neurologiche:

☐ Meningite

☐ Sclerosi multipla

☐ Epilessia

☐ Ictus

☐ Altre patologie cerebrovascolari

☐ Demenza

☐ Altre patologie neurologiche

Patologie psichiatriche o psicologiche:

☐ Ansia

☐ Depressione

☐ Trauma emotivo

☐ Stress eccessivo

Disturbi del sonno:

☐ Difficoltà ad addormentarsi

☐ Difficoltà a rimanere addormentato/a

Patologie cardiovascolari:

☐ Pressione bassa

☐ Pressione alta

☐ Infarto miocardico (attacco di cuore)

Patologie del sistema endocrino e metabolico

☐ Disfunzioni tiroidee

☐ Diabete

☐ Iperinsulinemia

☐ Colesterolo alto

Patologie reumatologiche e immunomediate:
☐ Artrite reumatoide
☐ Lupus eritematoso sistemico
Patologie otorinolaringoiatriche:
☐ Sinusite cronica
☐ Setto nasale deviato
Patologie infettive:
☐ Sifilide
☐ HIV
☐ Malattia di Lyme (borreliosi)
Altre patologie:
☐ Anemia
☐ Instabilità o altri disturbi dell'equilibrio
☐ Reflusso acido/gastroesofageo
☐ Bolo isterico
☐ Altro, specificare _____
☐ Nessuna patologia

A17	L'acufene, o tinnito, si riferisce ad una percezione di suoni in testa o nelle orecchie (come fischi o ronzii) in assenza di stimoli esterni.
	Nel corso dell'ultimo anno, ha mai avvertito tale sensazione nella testa, in una o entrambe le orecchie per più di 5 minuti?

☐ Sì, quasi sempre o sempre
☐ Sì, buona parte del tempo
☐ Sì, qualche volta
☐ No, non nell'ultimo anno
☐ No, mai
☐ Non so

Grazie per aver completato la sezione A. Se ha risposto "Si" alla domanda A17, per favore proceda alla sezione B. Se ha risposto "No" oppure "Non so" alla domanda A17, può considerare completato il questionario. Grazie per aver partecipato a questo studio.

SEZIONE B. CARATTERISTICHE DELL'ACUFENE

La ringraziamo per aver completato la sezione A. La preghiamo di indicare, per le domande seguenti, la risposta che meglio descrive il suo acufene e la relazione del suo acufene con altre patologie. In alcuni casi, è possibile selezionare più di una opzione.

B1	Quanto spesso soffre di acufene in media?

☐ Ogni giorno o quasi ogni giorno
☐ Circa una volta alla settimana
☐ Circa una volta al mese
☐ Una volta nel corso di più mesi
☐ Una volta all'anno

B2	Quale tra le seguenti opzioni descrive meglio l'andamento del suo acufene nel corso di una giornata?

☐ Costante: lo avverto sempre o abitualmente in una stanza silenziosa
☐ Intermittente: "va e viene", non lo sento sempre in una stanza silenziosa

B3	Quando è iniziato il suo acufene?

_ _ mesi
_ _ anni

☐ Non so

B4	Nel corso dell'ultimo anno, quanto il suo acufene la preoccupa, infastidisce o disturba, quando è al suo peggio?

☐ Fortemente
☐ Abbastanza
☐ Leggermente
☐ Per niente
☐ Non so

Se ha risposto "Per niente" oppure "Non so", per favore proceda alla domanda B6.

B5	Quanto tempo fa l'acufene ha iniziato a darle fastidio?

_ _ mesi
_ _ anni

☐ Non so

B6	In genere, i pazienti che soffrono di acufene lo percepiscono come un solo tipo di suono, ma alcuni sentono suoni diversi. Il suo acufene si manifesta con un solo tipo di suono, o con suoni differenti?

☐ Un suono ☐ Più di un tipo di suoni diversi

Nel caso in cui lei percepisca più di un solo tipo di suono, per favore si riferisca nelle prossime domande al tipo di acufene che le da più fastidio.

B7	Come è iniziato il suo acufene?

☐ Gradualmente ☐ All'improvviso ☐ Non so

B8	Se ha riferito una delle patologie/interventi di cui sopra, alle domande A9, A10, A11, A12, A13, A15 o A16, la preghiamo di riportarle qui, e di scrivere accanto a ciascuna se si sono manifestate PRIMA, DOPO o ALL'INCIRCA NELLO STESSO PERIODO dell'insorgenza dell'acufene.

_____
_____
_____
_____

B9	Indichi se l'insorgere dell'acufene è stato legato a uno degli eventi seguenti (è possibile selezionare più di una opzione):

☐ Esposizione a suoni forti
☐ Cambiamento nella percezione uditiva
☐ Cambiamento di pressione ambientale (per esempio durante un volo in aereo o durante un'immersione subaquea)
☐ Influenza, raffreddore o altra infezione
☐ Sensazione di orecchie tappate (aumentata pressione auricolare con ovattamento dei suoni)
☐ Stress
☐ Trauma cranico
☐ Trauma al collo (per esempio, colpo di frusta)
☐ Altro, specificare _____

☐ Nessuno

B10	Nel periodo in cui si è manifestato per la prima volta l'acufene, stava assumendo uno o più dei farmaci elencati?

E' possibile selezionare più di una opzione.

☐ Aspirina

☐ Antidolorifici, specificare _____

☐ Steoroidi orali, specificare _____

☐ Antibiotici, specificare _____

☐ Antidepressivi, specificare _____

☐ Chinino (per dolori muscolari o malaria)

☐ Diuretici, specificare _____

☐ Altri farmaci, specificare quali _____

☐ Nessuno

☐ Non so

B11	Crede che una o più delle condizioni mediche elencate sopra, o altre, siano legate all'insorgenza del suo acufene?

Può riportare 3 delle condizioni sopra elencate. Per favore, scelga quelle più rilevanti.

☐ No

☐ Sì, specificare _____

B12	L'intensità del suo acufene è stabile, o varia durante la giornata?

☐ Stabile

☐ Varia ogni tanto

☐ Varia continuamente

☐ Non so

B13	Che suono produce il suo acufene?

☐ Tonale

☐ Come un rumore

☐ Musicale

☐ Come dei grilli

☐ Altro, specificare _____

B14	La preghiamo di descrivere l'altezza del suono del suo acufene:

☐ Alto

☐ Medio

☐ Basso

☐ Non so

B15	Dove percepisce l'acufene?

☐ Orecchio destro

☐ Orecchio sinistro

☐ Entrambe le orecchie, ma più intensamente nel destro

☐ Entrambe le orecchie, ma più intensamente nel sinistro

☐ Entrambe le orecchie, con la stessa intensità

☐ Dentro la testa

☐ Altro, specificare _____

☐ Non so

B16	Descriverebbe il Suo acufene come un suono ritmico?

☐ No

☐ Si, segue il battito cardiaco (questo si può verificare prendendo il battito cardiaco nel momento in cui si manifesta l'acufene)

☐ Si, segue il ritmo respiratorio

☐ Si, segue il movimento di testa, collo, mandibola o muscoli facciali

☐ Altro, specificare _____

B17	E' mai capitato che un medico sentisse il suo acufene?

☐ Si ☐ No

B18	Il suo acufene è ridotto da uno o più dei seguenti fattori? (E' possibile selezionare più di una opzione)	B19	Il suo acufene è accresciuto da uno o più dei seguenti fattori? (E' possibile selezionare più di una opzione)

☐ Ambiente molto silenzioso	☐ Ambiente molto silenzioso
☐ Suoni a bassa intensità	☐ Suoni a bassa intensità
☐ Suoni ad alta intensità	☐ Suoni ad alta intensità
☐ Movimenti della testa	☐ Movimenti della testa
☐ Stringere i denti o muovere la mandibola	☐ Stringere i denti o muovere la mandibola
☐ Fare pressione su testa, collo, o area intorno all'orecchio	☐ Fare pressione su testa, collo, o area intorno all'orecchio
☐ Fare un sonnellino	☐ Fare un sonnellino
☐ Buona qualità del sonno	☐ Fare un sonno ristoratore
☐ Guidare	☐ Guidare
☐ Essere stressato o ansioso	☐ Essere stressato o ansioso
☐ Essere rilassato	☐ Essere rilassato
☐ Bere alcolici	☐ Bere alcolici
☐ Bere caffè	☐ Bere caffè
☐ Medicinali	☐ Medicinali
☐ Utilizzare supporti acustici	☐ Utilizzare supporti acustici
☐ Altro. Per favore, specificare _____ _____	☐ Altro. Per favore, specificare _____ _____
☐ Nessuno	☐ Nessuno

B20	Nel corso dell'ultimo anno, si è rivolto al suo medico di famiglia, o a un professionista sanitario presso una clinica o un ospedale, per i problemi legati a questi suoni in testa o nelle orecchie?

☐ Sì, per un totale di 5 o più visite

☐ Sì, per un totale di 2-4 visite

☐ Sì, solo una volta

☐ No, mai

☐ Non so

B21	In questo momento, sta ricevendo uno o più dei seguenti trattamenti/terapie per l'acufene? E' possibile selezionare più di una opzione.

☐ Trattamento psichiatrico

☐ Trattamento psicologico

☐ Trattamento audiologico

☐ Fisioterapia

☐ Auto-trattamento (e.g. integratori alimentari, gruppi di supporto, tecniche di

rilassamento)

☐ Altro. Per favore specificare _____

☐ Nessun trattamento

B22	Ritiene che una delle condizioni mediche elencate sopra possa essere legata a periodi di intensificazione dell'acufene?

Può riportare 3 delle condizioni sopra elencate. Per favore, scelga quelle più rilevanti.

☐ No

☐ Si, Per favore, specificare _____

Grazie per aver partecipato a questo sondaggio.

HOSPITALES DE LAS NIEVES DE GRANADA

Fecha | Nombre | Apellidos DE | DNI

...OADP...

...nos a
...siguien
...o actu...

...a de s... SI · A VECES · NO

...cuesta... SI · A VECES · NO

SI · A VECES · NO

SI · A VECES · NO

...el acúf... SI · A VECES · NO

...? SI · A VECES · NO

...io por SI · A VECES · NO

...insoluc... SI · A VECES · NO

9F ¿Interfiere su acúfeno en su vida social (salir a cenar, al cine)? SI · A VECES · NO

Meseta	125	250	500	1000	2000	4000	8000
V. aérea							
V. ósea							

Meseta	125	250	50
V. aérea			
V. ósea			

SI · A VECES · NO

11C ¿Cree que tiene una enfermedad incurable? SI · A VECES · NO

12F ...a? SI · A VECES · NO

13F ¿Interfiere su acúfeno en su trabajo o tareas del hogar? SI · A VECES · NO

Acufenometria	Derecho	Izquierdo
Frecuencia (Hz)		
Intensidad (dB SL)		
I. Enmascaramiento (dB SL)		
Inhibición residual		

Gelle
Weber
Rinne
Otro:

...eno? SI · A VECES · NO

15F ¿Tiene dificultades para leer por culpa de su acúfeno? SI · A VECES · NO

16E ¿Se encuentra uste... SI · A VECES · NO

17E ¿Cree que su acú... ...nterfiere en su relación con la familia o amigos? SI · A VECES · NO

18F ¿Es difícil, para usted, fijar su atención en cosas distintas a su acúfeno? SI · A VECES · NO

Caracterís...
Modelo del aud...
Auriculares
Fecha de la últi... calibración

19C ¿Cree que su acúfeno es incontrolable SI · A VECES · NO

20F ¿Se siente a menudo cansado por culpa de su acúfeno? SI · A VECES · NO

21E ¿Se siente deprimido por culpa de su acúfeno? SI · A VECES · NO

22E ¿Se siente ansioso por culpa de su acúfeno? SI · A VECES · NO

23C ¿Cree que su problema de acúfenos le desborda? SI · A VECES · NO

24F ¿Empeora su acúfeno cuando tiene estrés? SI · A VECES · NO

25E ¿Se siente usted inseguro por culpa de su acúfeno? SI · A VECES · NO

(charts: dB HL axis, kHz .125 .25 .5 .75 1 1.5 2 3 4 6 8 10 12.5 16 20 / 9 11.2 14 18; % Aciertos vs dB HL 0–25)

The Italian translation of the "Tinnitus Handicap Inventory" by Newman CW, Jacobson GP & Spitzer JB (1996).

Tinnitus Handicap Inventory (THI)	4	2	0
L'acufene le provoca difficoltà di concentrazione?	Sì	Qualche volta	No
L'intensità dell'acufene le provoca difficoltà nel comprendere le parole?	Sì	Qualche volta	No
L'acufene la rende infelice?	Sì	Qualche volta	No
L'acufene la fa sentire confuso/confusa?	Sì	Qualche volta	No
È disperato/disperata per il suo acufene?	Sì	Qualche volta	No
Si lamenta molto per il suo acufene?	Sì	Qualche volta	No
Ha problemi ad addormentarsi la notte a causa del suo acufene?	Sì	Qualche volta	No
Ha la sensazione che non potrà liberarsi dal suo acufene?	Sì	Qualche volta	No
L'acufene interferisce con le sue attività sociali? (ad esempio andare al cinema, a pranzo)	Sì	Qualche volta	No
Si sente frustrato/frustrata dal suo acufene?	Sì	Qualche volta	No
Crede che l'acufene le provochi un terribile disagio?	Sì	Qualche volta	No
L'acufene le crea difficoltà nella vita di tutti i giorni?	Sì	Qualche volta	No
L'acufene interferisce nel suo lavoro o nei lavori domestici?	Sì	Qualche volta	No
Crede di esser spesso irritabile a causa del suo acufene?	Sì	Qualche volta	No
La sconvolge il suo acufene?	Sì	Qualche volta	No
Crede che l'acufene provochi stress nelle relazioni con amici e parenti?	Sì	Qualche volta	No
Trova difficoltoso focalizzare l'attenzione su qualcosa che non sia l'acufene?	Sì	Qualche volta	No
Trova difficoltoso leggere por il suo acufene?	Si	Qualche volta	No
Le sembra di non aver il controllo del suo acufene?	Sì	Qualche volta	No
Si sente stanco/stanca a causa del suo acufene?	Sì	Qualche volta	No
Si sente depresso/depressa a causa del suo acufene?	Sì	Qualche volta	No
L'acufene le provoca ansia?	Sì	Qualche volta	No
Sente di non poter convivere ancora a lungo con il suo acufene?	Sì	Qualche volta	No
L'acufene peggiora quando lei è sotto stress?	Sì	Qualche volta	No
L'acufene le provoca insicurezza?	Sì	Qualche volta	No

ESCALA HOSPITALARIA DE ANSIEDAD Y DEPRESIÓN

El siguiente cuestionario ha sido confeccionado para ayudar a saber cómo se siente usted afectiva y emocionalmente. No es preciso que preste atención a los números que aparecen a la izquierda.
Lea cada pregunta y marque la que usted considere que coincide con su propio estado emocional en la última semana.
No es necesario que piense mucho tiempo cada respuesta; en este cuestionario las respuestas espontáneas tienen más valor que las que se piensan mucho.

A.1. Me siento tenso/a o nervioso/a:

3) Casi todo el día ☐   2) Gran parte del día ☐   1) De vez en cuando ☐   0) Nunca ☐

D.1. Sigo disfrutando de las cosas como siempre:

0) Ciertamente igual que antes ☐   1) No tanto como antes ☐   2) Solamente un poco ☐   3) Ya no disfruto con nada ☐

A.2. Siento una especie de temor como si algo malo fuera a suceder:

3) Sí, y muy intenso ☐   2) Sí, pero no muy intenso ☐   1) Sí, pero no me preocupa ☐   0) No siento nada de eso ☐

D.2. Soy capaz de reírme y ver el lado gracioso de las cosas:

0) Igual que siempre ☐   1) Actualmente algo menos ☐   2) Actualmente mucho menos ☐   3) Actualmente en absoluto ☐

A.3. Tengo la cabeza llena de preocupaciones:

3) Casi todo el día ☐   2) Gran parte del día ☐   1) De vez en cuando ☐   0) Nunca ☐

D.3. Me siento alegre:

3) Nunca ☐   2) Muy pocas veces ☐   1) En algunas ocasiones ☐   0) Gran parte del día ☐

A.4. Soy capaz de permanecer sentado/a, tranquilo/a y relajado/a:

0) Siempre ☐   1) A menudo ☐   2) A veces ☐   3) Nunca ☐

D.4. Me siento lento/a y torpe:

3) Gran parte del día ☐   2) A menudo ☐   1) A veces ☐   0) Nunca ☐

A.5. Experimento una desagradable sensación de "nervios y hormigueos" en el estómago:

0) Nunca ☐   1) Sólo en algunas ocasiones ☐   2) A menudo ☐   3) Muy a menudo ☐

D.5. He perdido el interés por mi aspecto personal:

3) Completamente ☐   2) No me cuido como debería hacerlo ☐

1) Es posible que no me cuide como debiera ☐   0) Me cuido como siempre lo he hecho ☐

A.6. Me siento inquieto/a como si no pudiera parar de moverme:

3) Realmente mucho ☐   2) Bastante ☐   1) No mucho ☐   0) En absoluto ☐

D.6. Espero las cosas con ilusión:

0) Como siempre ☐   1) Algo menos que antes ☐   2) Mucho menos que antes ☐   3) En absoluto ☐

A.7. Experimento de repente sensaciones de gran angustia o temor:

3) Muy a menudo ☐   2) Con cierta frecuencia ☐   1) Raramente ☐   0) Nunca ☐

D.7. Soy capaz de disfrutar con un buen libro o con un buen programa de radio o televisión:

0) A menudo ☐   1) Algunas veces ☐   2) Pocas veces ☐   3) Casi nunca ☐

Puntuación HAD-A: 1) < 7 ☐   2) 8-10 ☐   3) > 11 ☐   **Puntuación HAD-D:** 1) < 7 ☐   2) 8-10 ☐   3) > 11 ☐

Hospital Anxiety and Depression Scale (H. A. D. S.)

Indichi per ogni affermazione la risposta più vicina al suo stato emozionale:

A 1 Mi sono sentito teso e molto nervoso:		D. 4 Mi sono sentito rallentato nei movimenti:	
3 Per la maggior parte del tempo		3 Quasi sempre	
2 Per molto tempo		2 Molto spesso	
1 A volte		1 A volte	
0 Mai		0 Mai	
D 1 Sono riuscito ancora a provare piacere per le cose che ho sempre fatto volentieri:		A 5 Mi sono sentito nervoso, come con un senso di tensione allo stomaco:	
0 Proprio come una volta		0 Mai	
1 Non proprio come una volta		1 A volte	
2 Solo in parte		2 Piuttosto spesso	
3 Per niente		3 Molto spesso	
A 2 Ho provato un sentimento di paura come se potesse accadere qualcosa di terribile:		D. 5 Ho perso interesse per il mio aspetto fisico:	
3 Sicuramente e in maniera intensa		3 Completamente	
2 Si, ma in maniera non troppo intensa		2 Non me ne prendo cura quanto dovrei	
1 Un po' ma non da preoccuparmene		1 Forse non me ne prendo cura abbastanza	
0 Per niente		0 Me ne prendo cura come al solito	
D 2 Sono riuscito a ridere e a vedere il lato divertente delle cose:		A 6 Mi sono sentito irrequieto e incapace di stare fermo:	
0 Proprio come ho sempre fatto		3 Moltissimo	
1 Non proprio come un tempo		2 Molto	
2 Sicuramente non come un tempo		1 Non molto	
3 Per niente		0 Per niente	
A 3 Mi sono venuti in mente pensieri preoccupanti:		D. 6 Penso al futuro con ottimismo:	
3 Per la maggior parte del tempo		0 Così come ho sempre fatto	
2 Per molto tempo		1 Un po' meno di una volta	
1 A volte, non troppo spesso		2 Sicuramente meno di una volta	
0 Solo in qualche occasione		3 Per niente	
D. 3 Mi sono sentito di buon umore:		A 7 Mi sono venute improvvise crisi di panico:	
3 Mai		3 Molto spesso	
2 Raramente		2 Piuttosto spesso	
1 A volte		1 Non molto spesso	
0 Per la maggior parte del tempo		0 Mai	
A 4 Ho potuto sedermi sentendomi rilassato e a mio agio:		D. 7 Ho provato piacere leggendo un buon libro o seguendo la radio o la televisione:	
0 Sempre		0 Spesso	
1 Spesso		1 A volte	
2 Qualche volta		2 Non di frequente	
3 Mai		3 Molto raramente	

Test de hipersensibilidad al sonido de Nelting

Conteste a las siguientes afirmaciones poniendo una X en la casilla que corresponda.

	Nunca	En ocasiones	Frecuente-mente	Siempre
Ciertos ruidos, que antes no me molestaban, ahora me provocan miedo				
Me preocupa la idea de que nunca voy a ser capaz de acostumbrarme a estos sonidos fuertes y desagradables				
Cuando tengo alrededor ruidos fuertes o desagradables no puedo escuchar o prestar atención				
Tengo problemas con mi pareja o familia por mi mayor sensibilidad a los sonidos				
Ante la presencia de ciertos sonidos, tengo la necesidad de manifestarlo o decírselo a los demás				
Tengo miedo a los ruidos intensos				
Pienso que la hipersensibilidad a los sonidos que tengo me ha arruinado la vida				
Cuanto tengo muchos ruidos alrededor no oigo ni entiendo nada				
Algunas personas me evitan porque no soporto ruidos fuertes o desagradables				
Los sonidos fuertes o desagradables me provocan enfado				
Tengo dolor de oídos cuando hay ruidos intensos o desagradables				
Pienso que voy a ser incapaz de enfrentarme a la vida diaria si persiste mi hipersensibilidad a los ruidos				
Cuando hay ruidos o sonidos intensos y desagradables me retiro o me retraigo inmediatamente				
Tengo miedo porque los ruidos fuertes o desagradables deterioren mi audición				
Desde que tengo esta hipersensibilidad a los sonidos ya no disfruto de la música				

Questionario Nelting d'iperacusia (Versione Italiana)

Risponda alle siguenti domande

	MAI	QUALCHE VOLTA	SPESSO	SEMPRE
1. Suoni, che prima non mi disturbavano, ora mi spaventano Sono preoccupato per l'idea di non riuscire mai ad abituarmi a questi suoni forti e sgradevoli.				
2. Sono preoccupato di non riuscire mai ad abituarmi a suoni forti e sgradevoli				
3. Non riesco ad ascoltare a lungo quando ci sono rumori forti e sgradevoli intorno a me				
4. A causa della mia ipersensibilità ai suoni, c'è tensione tra me e il mio compagno o con la mia famiglia				
5. Devo evitare alcuni suoni				
6. Ho paura dei rumori intensi				
7. Penso che la mia ipersensibilità ai suoni ha rovinato la mia vita				
8. Quando ci sono tanti rumori intorno a me, non capisco nulla				
9. Alcune persone mi evitano perche non sopporto souni intensi o sgradevoli				
10. Sono infastidito dai suoni che sono troppo forti o sgradevoli per me				
11. Rumori forti/sgradevoli mi causano dolore fisico alle orecchie				
12. Penso di non poter far fronte alla mia vita quotidiana se la mia ipersensibilità ai suoni prosegue così male				
13. Quando ci sono suoni intensi oppure sgradevoli, mi ritiro immediatamente				
14. Ho paura che i souni forti/sgradevoli danneggino il mio udito				
15. Da quando è cominciata la mia ipersensibilità ai suoni, non apprezzo più la musica				

The Italian translation of the hyperacusis questionnaire by Khalfa S. et al., 2002.

Questionario sull'iperacusia

Cognome, Nome: _____ Data: _____

Sesso: [M] [F] Età: _____

Professione:_____

Città di residenza:_____

Telefono fisso / mobile:_____

È stato o è esposto al rumore?_____

Tollera il rumore meno bene di qualche anno fa?_____

Ha mai avuto problemi di udito? Se sì di che tipo? _____

	No	Raramente	Spesso	Sempre
1. Ha l'abitudine ad usare tappi o cuffie per ridurre la percezione del rumore (non consideri l'utilizzo di protezioni auricolari durante situazioni di anormali od elevati rumori)?				
2. Le riesce difficile ignorare i suoni circostanti in situazioni quotidiane?				
3. Ha difficoltà a leggere in ambienti rumorosi?				
4. Ha difficoltà a concentrarsi in situazioni rumorose?				
5. Ha difficoltà a seguire la conversazione in ambienti rumorosi?				
6. Qualcuno le ha detto che tollera poco il rumore o alcuni suoni?				
7. È particolarmente sensibile o disturbato dai rumori della strada?				
8. Trova il rumore sgradevole in alcune situazioni sociali (night club, pub, bar, concerti, rinfreschi, spettacoli pirotecnici)?				
9. Quando le propongono qualcosa (uscire, andare al cinema, andare ad un concerto) pensa immediatamente al rumore al quale potrà essere esposto?				
10. Rinuncia mai ad inviti o ad uscire a causa del rumore a cui potrebbe essere esposto?				
11. Il rumore o particolari suoni la disturbano maggiormente in un luogo silenzioso piuttosto che in presenza di un leggero rumore di fondo?				
12. Lo stress e la stanchezza riducono la sua capacità di concentrazione in				
13. La sua capacità di concentrazione in presenza di rumore diminuisce verso la fine della giornata?				
14. Il rumore o alcuni suoni le causano stress od irritabilità?				

CONSENTIMIENTO INFORMADO – CONSENTIMIENTO POR ESCRITO DEL PACIENTE

Yo (Nombre y Apellidos):………………………………………………………………………………………………….,

con DNI…………………………..

- He leído el documento informativo que acompaña a este consentimiento (Información al Paciente)

He podido hacer preguntas sobre el estudio y he recibido suficiente información sobre el estudio

Caracterización clínica y genética molecular de pacientes con migraña vestibular

- He hablado con el profesional sanitario informador: Elisheba Haro Hernández

- Comprendo que mi participación es voluntaria y soy libre de participar o no en el estudio.

- Se me ha informado que todos los datos obtenidos en este estudio serán confidenciales y se tratarán conforme establece la Ley Orgánica 3/2018 de Protección de Datos Personales.

- Se me ha informado de que la donación/información obtenida sólo se utilizará para los fines específicos del estudio.

- **Deseo** ser informado/a de mis datos genéticos y otros de carácter personal que se obtengan en el curso de la investigación, incluidos los descubrimientos inesperados que se puedan producir, siempre que esta información sea necesaria para evitar un grave perjuicio para mi salud o la de mis familiares biológicos.

Si   No

Comprendo que puedo retirarme del estudio:

- Cuando quiera
- Sin tener que dar explicaciones
- Sin que esto repercuta en mis cuidados médicos

Presto libremente mi conformidad para participar en el proyecto "Caracterización clínica y genética molecular de pacientes con migraña vestibular"

Firma del paciente
(o representante legal en su caso)

Firma del profesional
sanitario informador

Nombre y apellidos:………………………
DNI ………………………………….
Fecha: …………………………

Nombre y apellidos: ……………………..
DNI……………………………
Fecha: ………………………..

FOGLIO INFORMATIVO e CONSENSO INFORMATO

CONSENSO INFORMATO – CONSENSO SCRITTO DEL PAZIENTE

Il sottoscritto (Nome e Cognome) ……………………………………………………………………………, con n° Carta di Identità …………………………..

- Ho letto il documento informativo che allegato a questo consenso (Scheda informativa per il Paziente)

- Ho potuto porre le domande sullo studio e ho ricevuto sufficienti informazioni sullo studio:

   Caratterizzazione clínica e genetica molecolare dei pazienti con emicrania vestibolare

- Ho parlato con il professionista sanitario: Elisheba Haro Hernández

- Comprendo che la mia participazione è volontaria e sono libero di participare o meno allo studio.

- Sono stato informato che tutti i miei dati ottenuti in questo studio saranno riservati e saranno trattati come stabilito dalla Ley Orgánica 3/2018 de Protección de Datos Personales (Spagna).

- Sono stato informato che le informazioni ottenute saranno utilizzate solo per   s per i fine specifici dello studio.

- Desidero essere informato/a dei miei dati genetici e di altri dati dati personali ottenuti nel corso della ricerca, compresi i risultati imprevisti che potrebbero riscontrarsi , ogni tal volta che tali informazioni siano necessari per evitare gravi danni alla mia salute o a quella dei miei parenti biologici
                                        Si   No

Prendo atto che posso ritirarmi dallo studio :

- Quando lo desidero
- Senza spiegazioni
- Senza che questo abbia impatto sulla mia assistenza medica

   Presto liberamente il mio consenso a participare al progetto "Caratterizzazione clínica e genética molecolare dei pazienti con emicrania vestibolare"

Firma del paziente   Firma del Professionista Sanitario
(o del rappresentante legale,)   Informatore

Nome e Cognome:………………   Nome e Cognome: ………….....
N° Carta di Identità ………..   N° Carta di identità ....
Data: ………………………….   Data: …………………………......

CONSENTIMIENTO INFORMADO PARA INVESTIGACION CLINICA CON MUESTRAS BIOLOGICAS – HOJA DE INFORMACIÓN AL PACIENTE

Caracterización clínica y genética molecular de pacientes con migraña vestibular

Antes de proceder a la firma de este consentimiento informado, lea atentamente la información que a continuación se le facilita y realice las preguntas que considere oportunas.

Su médico le ha diagnosticado una migraña vestibular (MV). Es una enfermedad crónica que produce crisis recurrentes de vértigo y cefalea migrañosa con o sin aurea, que pueden presentarse simultáneamente o en momentos temporales diferentes. Actualmente, no existe un tratamiento para su curación definitiva, sin embargo, los síntomas pueden controlarse y prevenirse mediante algunos fármacos y evitando los desencadenantes que provocan las crisis migrañosas, aunque su eficacia no está demostrada. El objetivo de este estudio es contribuir a identificar los genes de la MV mediante un estudio que incluye el análisis de familias donde existan tres casos diagnosticados de MV.

Le ofrecemos la posibilidad de participar **voluntariamente** en un estudio que nos permita determinar el componente genético que existe en el desarrollo de la MV, y que nos sirvan para obtener una mejor comprensión de la enfermedad con la idea de desarrollar diagnósticos personalizados y decisiones terapéuticas que mejoren el manejo clínico de los pacientes.

El estudio ha sido autorizado por el Comité de Ética e Investigación Clínica del Hospital. Esta información es para explicarle en que consiste el estudio. La participación es totalmente voluntaria.

En este estudio le pediremos que nos facilite una **muestra de sangre periférica** que formará parte de un banco de muestras de pacientes, que se almacenará hasta la finalización del proyecto. Su médico le realizará una única extracción en el punto inicial de su reclutamiento en el proyecto. Además, se le pedirá una muestra de sangre de sus otros familiares afectos.

Las muestras se recogerán de forma prospectiva y serán obtenidas expresamente para las investigaciones de este estudio. Las muestras de sangre serán procesadas para obtener ADN (que contiene información genética estructural). Dichas muestras serán conservadas durante 4 años en el Centro de Genómica e Investigación Oncológica Pfizer/Universidad de Granada/Junta de Andalucía (GENyO). La persona responsable de custodiar las muestras es el Dr. José Antonio López Escámez, Investigador Principal del Grupo de Otología y Otoneurología CTS495- Genómica de Trastornos vestibulares (http://www.genyo.es/content/ver-grupos-de-investigacion?id=5).

Las muestras serán codificadas, disociadas y la identificación de las mismas no será posible durante su utilización en el laboratorio. Estas muestras forman parte de una colección registrada en la Plataforma y Registro Nacional de Biobancos (sección colecciones), en cumplimiento con el Real Decreto 1716/2011, de 18 de noviembre, por el que se establecen los requisitos básicos de autorización y funcionamiento de los Biobancos con fines de investigación biomédica y del tratamiento de las muestras biológicas de origen humano con el Nº de registro 2014999E000810.

El acceso y análisis genético de las muestras se realizará por el personal autorizado que forma parte de este proyecto. Finalizados los análisis del presente proyecto, las muestras no se almacenarán en ningún biobanco para estudios posteriores.

El donante/paciente puede retirarse del estudio cuando así lo manifieste, sin dar explicaciones y sin que esto repercuta en sus cuidados médicos. Todos los datos de carácter personal, obtenidos en este estudio son confidenciales y se tratarán conforme a la Ley Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales.

La donación/información obtenida se utilizará exclusivamente para los fines específicos de este estudio. Si Vd. desea recibir la información de los resultados del estudio, tiene derecho a conocerla cuando este concluya, previa petición por escrito de la misma.

Riesgos de la investigación para el donante/paciente:
No existe ningún riesgo adicional por la donación de sangre. Si requiere información adicional se puede poner en contacto con el personal de la Unidad de Gestión Clínica de Otorrinolaringología de su Hospital.

CONSENSO INFORMATO PER L'INVESTIGAZIONE CLINICA CON CAMPIONI BIOLOGICI – SCHEDA INFORMATIVA PER IL PAZIENTE

Caratterizzazione clínica e genetica molecolare dei pazienti con emicrania vestibolare

Prima di procedere alla firma di questo consenso informato, legga attentamente l'informativa fornita e porga le domande che riterrà più opportune

Il Suo medico ha diagnosticato una emicrania vestibolare(EV). È una malattia cronica che produce crisi ricorrente di vertigine e emicrania con oppure senza aurea, che si possono presentare contemporaneamente oppure in momenti diversi. Al momento, non esiste un trattamento che porti ad una guarigione definitiva , tuttavia, i sintomi possono essere controllati e prevenuti attraverso alcuni farmaci e evitando i fattori scatenanti che provocano gli attacchi di emicrania, sebbene la loro efficacia non sia stata demostrata. L'obiettivo di questo studio è quello di contribuire a identificare i geni della EV attraverso uno studio che include le analisi di famiglie dove sono presenti tre casi diagnosticati de EV.

Le offiriamo l'opportunità di participare **volontariamente** ad uno studio che ci permetta di determinare la componente genetica che esiste nello sviluppo della EVe che ci aiuti ad ottenere una migliore comprensione della mallatia con l'idea di sviluppare una diagnosi personalizzata e trattamenti che migliorino la gestione clinica dei pazienti.

Lo studio è stato autorizzato dal Comitato etico e di ricerca clinica dell'ospedale Clinico San Cecilio di Granada (Spagna). Queste informazioni servono per spiegare in cosa consiste lo studio .

Se è un candidato, le chiederemo di fornirci un campione di saliva o sangue che sará conservato in una banca di campioni di pazienti fino alla fine del progetto. Il suo medico eseguirà un'unica estrazione al inizio del progetto. Inoltre, le verrà chiesto un campione di sangue degli altri Suoi parenti affetti. I campioni saranno raccolti prospetticamente e saranno ottenuti espressamente per le indagini di questo studio. I campione di sangue/saliva saranno usati per ottenete DNA (che contiene l'informazione genetica strutturale). Questi campioni saranno conservati per 4 anni presso il Centro de Genómica e Investigación Oncológica Pfizer/Universidad de Granada/Junta de Andalucía (GENyO). Le persona responsabile della conservazione dei campioni è il Dr. José Antonio López Escámez, Investigatore Principale dil Grupo de Otología y Otoneurología CTS495- Genómica de Trastornos vestibulares (http://www.genyo.es/content/ver-grupos-de-investigacion?id=5).

I campioni saranno codificati, dissociati e l'identificazione non sará posibile durante il loro utilizzo in laboratorio . Tali campioni fanno parte di una collezione registrata presso la "Plataforma y Registro Nacional de Biobancos" (sección colecciones), in conformità con il "Real Decreto 1716/2011", del 18 Novembre,che stabilisce i requisiti minimi per l' autorizzazione e funzionamento delle Biobanche ai fini dell' investigazione biomedica e il trattamento dei campioni biologici di origine umana con il Nº di registrazione 2014999E000810.

L'accesso e l'analisi genetica dei campioni saranno effettuati da personale autorizzato che fa parte di questo progetto.Una volta ultimato il progetto, i campioni non saranno conservati in alcuna Biobanche per future investigazioni.

Il paziente può ritirarsi dallo studio quando desidera, senza dare spiegazioni e senza che questo abbia impatto sulla sua assistenza medica. Tutti i dati personali ottenuti durante lo istudio sono riservati e si tratteranno in conformità con la Legge Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales.

Le informazioni ottenute saranno utilizzate esclusivamente per questo studio. Se desiderasse ricevere informazioni sui risultati dello, ha diritto a riceverli al termine dello studio,previa richiesta scritto.

Rischi dell' investigazione per il donante/paziente:
Non esiste rischio aggiuntivo per la donazione della sangue/saliva. Se desiderasse ricevere più informazioni può mettersi in contatto con il personale dell'Unità d'Audiologia dell'Ospedale.