REVIEW ARTICLE

Hereditary Hearing Loss: Genetic Counselling

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Abstract The aim of this review is to provide an updated overview of hereditary hearing loss, with special attention to the etiological diagnosis of sensorineural hearing loss, the genes most frequently mutated in our environment, the techniques available for their analysis and the clinical implications of genetic diagnosis.

More than 60% of childhood sensorineural hearing loss is genetic. In adults, the percentage of hereditary hearing loss is unknown. Genetic testing is the highest yielding test for evaluating patients with sensorineural hearing loss. The process of genetic counselling is intended to inform patients and their families of the medical, psychological, and familial implications of genetic diseases, as well as the risks, benefits and limitations of genetic testing. The implementation of any genetic analysis must be always preceded by an appropriate genetic counselling process.

PALABRAS CLAVE
Consejo genético; Hipoacusia hereditaria; Sordera; Hipoacusia sensorineural; Hipoacusia sindrómica; Hipoacusia no sindrómica; GJB2

Hipoacusias hereditarias: asesoramiento genético

Resumen El objetivo de esta revisión es proporcionar una visión actualizada de las hipoacusias hereditarias, prestando especial atención al diagnóstico etiológico de las hipoacusias sensoriales, a los genes más frecuentemente mutados en nuestro medio, a las técnicas disponibles para su estudio y a las implicaciones clínicas del diagnóstico genético.

Al menos el 60% de las hipoacusias sensoriales infantiles tienen una causa genética. En los adultos desconocemos el porcentaje de hipoacusias hereditarias. Ante una hipoacusia sensorial, la prueba con un mayor rendimiento diagnóstico son los análisis genéticos. El proceso de consejo o asesoramiento genético tiene como fin informar al paciente y sus familiares de la probabilidad de presentar una enfermedad condicionada genéticamente, del riesgo de transmitirla, de las medidas de prevención y diagnóstico precoz disponibles, y de la posibilidad de llevar a cabo un estudio genético. La realización de cualquier análisis genético, siempre ha de venir precedida por el adecuado proceso de asesoramiento genético.

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Introduction

It is estimated that at least 60% of early-onset hearing loss or hypoaucis cases respond to a genetic cause, whilst the remaining 40% are attributed to environmental causes. The latter include prenatal infections (cytomegalovirus, herpesvirus, rubella, toxoplasmosis, etc.), postnatal infections (bacterial meningitis), foetal distress, hyperbilirubinemia or ototoxic drugs. These percentages have been changing in recent years in parallel with the technological development experienced by molecular biology. It is important to note that the presence of an environmental cause does not necessarily exclude the existence of an underlying genetic predisposition. The genetic analysis of patients with hearing loss due to environmental causes commonly identifies pathogenic mutations. This is true both in early-onset and late-onset hearing losses, where the interaction between environmental and genetic factors is even more complex. Therefore, caution is required when classifying hearing loss as environmental, since this may have important implications at the time of genetic counselling (for example, probability of occurrence of new cases in the family).

Genetic or hereditary hearing loss can be classified as conductive, perceptive or mixed; as syndromic or non-syndromic; and finally, as pre-lingual or post-lingual. Hearing loss is labelled as syndromic when associated with external ear malformations or manifestations in other organs or systems. By contrast, non-syndromic cases are not associated with other medical problems, although they may present abnormalities in the middle or inner ear. Approximately 30% of pre-lingual genetic hearing loss cases are syndromic (hypoaucis is an identifiable symptom in over 400 different syndromes). In the remaining 70% of cases, hearing loss is not associated with any other clinical manifestations (non-syndromic hearing loss). Among pre-lingual non-syndromic hearing loss cases, 80% are inherited following an autosomal recessive (AR) pattern, 18% follow an autosomal dominant (AD) pattern and the remaining 2% correspond to hearing loss with X-linked and mitochondrial genome inheritance. The percentages for each inheritance pattern in cases of post-lingual non-syndromic hearing loss are currently unknown. However, the percentage of families with an AD pattern is higher than the pre-lingual.

In order to classify non-syndromic hearing loss, the different loci (chromosomal regions where the genes associated with the disease are located) are designated as DFN (derived from Deafness), followed by a number related to the chronological order of their discovery. AD loci are known as DFN, AR loci are known as DFN and X-linked loci are known as DNX.

Genetic Characteristics of Hereditary Hypoaucis

When designing the study of hereditary hearing loss it is essential to be familiar with four classical genetic concepts which characterise them:

- Incomplete penetrance: only a certain percentage of individuals carrying the altered gene manifest the disease. For example, only 60% of individuals carrying a heterozygous mutation in the PAX3 gene (Waardenburg syndrome type 1 and type 3) develop hearing loss.  
- Variable expressivity: the severity of clinical manifestations differs between individuals carrying the same mutation. For example, individuals carrying a heterozygous mutation in the WFS1 gene may present moderate perceptive post-lingual hearing loss as the only symptom or else present other characteristic symptoms of Wolfram syndrome (diabetes mellitus and/or optic atrophy).  
- Genetic heterogeneity: mutations in different genes can cause the same clinical manifestation. In fact, there are at least 30 genes capable of causing pre-lingual non-syndromic hearing loss with an autosomal recessive pattern (GJB2, GJB6, OTOF, TECTA, MYO7A, etc.).  
- Allelic heterogeneity: a single mutation can give rise to different diseases. For example, the recurrent mutation 35delG in the GJB2 gene (connexin 26), can cause AR non-syndromic hearing loss (DFNB1), AD non-syndromic hearing loss (DFNA3) or a syndrome with cutaneous, vascular or thyroid manifestations.

Therefore, in the case of genetic hearing loss, incomplete penetrance, variable expressivity and genetic and allelic heterogeneity hinder the establishment of correlations between a specific mutation (genotype) and its clinical and audiometric manifestations (phenotype). This partly justifies the difficulty of integrating genetic counselling in daily clinical practice, despite its benefits for patients and their families.

Genetic Counselling

Genetic advice or counselling is defined as a communication process in which patients and/or their families are informed of the likelihood of developing a particular, genetically determined disease, the risk of transmitting it, its preventive measures, early diagnosis, and available treatment, as well as the possibility of conducting a genetic study, which is not always feasible or necessary.

During genetic counselling, it is important to note that some deaf people do not consider hearing loss as a 'deficit' and do not wish for their deafness to be considered as a disease to be treated or cured. Moreover, some deaf couples wish to have deaf children. Therefore, although the process of genetic counselling should always place its emphasis on respecting patient individuality and not being directive, these assumptions are particularly important in the case of deaf patients. It is also necessary to consider that hearing loss and its possible associated communication difficulties may represent a problem at the time of counselling.

Genetic studies in Spain are regulated by the Biosanitary Research Act 14/2007. This law establishes the need for a process of genetic counselling before and after the completion of any genetic test, as well as the need for specific informed consent. Therefore, the results of a genetic test must always be presented within an environment of genetic counselling. In this consultation, patients and their families should be informed about the ethical and social issues associated with genetic studies (confidentiality, implications for family, risk of social and labour discrimination, possibility...
of revealing false paternity, attitude with uncertain results, psychological implications, etc.).

Therefore, if following an appropriate clinical diagnosis there is suspicion that hearing loss could have a hereditary origin, patients should be referred to genetic counselling. This specialist consultation should be part of the multidisciplinary team responsible for managing deaf patients. It should integrate otolaryngologists, speech therapists, paediatricians, phoniatricians, clinical geneticists and, eventually, neurologists and ophthalmologists. This consultation, prior to discussion with the patient and/or their families about the possibility of conducting a genetic study, should attempt to characterise hearing loss as far as possible.

Aetiological Diagnosis of Sensorineural Hearing Loss

The aetiological diagnosis of a potentially genetic sensorineural hearing loss is complex and there is no standardised protocol for it. Furthermore, we must not forget that the boundary between environmental and genetic hearing loss is becoming increasingly difficult to establish. Examples of this are the identification of mutations in the GJB2 gene in allegedly environmental cases3, or in the OTOF gene in apparently sporadic cases (with no family history).7

In order to obtain an aetiological diagnosis it is necessary to accurately know the family and personal history, conduct a thorough physical examination and, when indicated, request additional radiological, biochemical or genetic studies. A correct coordination between the different members of the multidisciplinary team will avoid unnecessary duplication of diagnostic tests.

Family History

Building a good family tree is the starting point for adequate genetic counselling. Ideally, it should cover three generations and include, at least, audiological and otological examinations of first-degree relatives of the index case. Incomplete penetrance, variable expressivity and genetic and allelic heterogeneity should be taken into account when assessing a family tree, especially for genetic counselling of family members. It is important to remember that family history is dynamic, and therefore should be reassessed in subsequent reviews. The evaluation of a specific genealogy should always take into account the possibility of false paternities, adoptions or even the possibility of mutations arising de novo, establishing patients as first carriers in the family.8 The identification of a transmission pattern within a family represents a significant step for genetic counselling.

Personal History

A detailed medical history should include information on maternal health, pregnancy, childbirth and the postnatal period. Special attention should be paid to the use of potentially ototoxic drugs during pregnancy, including alcohol consumption. Infections should also be considered, especially bacterial meningitis. It is necessary to rule out disease in other organs or systems, especially neurological, ophthalmological or vestibular. Furthermore, in children it is important to note an adequate progression through various psychomotor development milestones.

Physical Examination

The first goal should be to classify hearing loss as syndromic or non-syndromic. This requires a thorough and systematic exploration, with attention to the characteristic signs of the most common syndromes (for example, auricular malformations, branchial cysts, white forelock, telecanthus, goitre, craniofacial and skeletal abnormalities, etc.).9

Complementary Studies

After an adequate auditive assessment (otoacoustic emissions, auditory evoked potentials, tympanometry, acoustic stapedial reflex and age-appropriate audiometry), the type of hearing loss and age of the patient should guide which tests to be requested. Possible studies to be considered include magnetic resonance imaging (MRI) and computed tomography (CT) scans, renal ultrasound, serologies (cytomegalovirus, toxoplasmosis, syphilis, rubella, etc.), blood and urine biochemical analyses, electrocardiogram, eye fundus and electroretinography.

Except for MRI and CT scans, the diagnostic usefulness of the other tests in previously unscreened patients is very low and their role in diagnostic protocols has not been adequately defined. However, we must not forget that they can be highly relevant in certain cases, such as detection of abnormalities in the electrocardiogram of patients with Jervell and Lange-Nielsen syndromes, hypothyroidism in a percentage of patients with Prendred syndrome, renal failure in Alström, Alport and branchio-oto-re nal syndromes, etc.

Inner ear MRI and temporal bone CT scans enable detection of inner ear malformations. As we shall see, some of these are correlated with the presence of mutations in certain genes (for example, Mondini aplasia, enlarged vestibular aqueduct, dilation of the internal auditory canal, etc.). However, the genetic heterogeneity typical of hearing loss limits their usefulness (for example, enlarged vestibular aqueduct, considered as a classic sign of mutations in the SLC26A4 gene, can be found relatively frequently in patients with mutations in the GJB2 gene).10

Currently, in children, genetic studies must always be the first step in the aetiological diagnosis of moderate to severe sensorineural non-syndromic hearing loss, except in specific cases where the probability of finding a radiological anomaly is relatively high (mild hearing loss, late-onset, progressive or fluctuating hearing loss and hearing loss with associated vestibular or craniofacial pathology).10

Genetic Studies

Whenever it is not possible to identify a clear cause through medical history or physical examination, genetic studies represent the diagnostic test with the highest
performance in non-syndromic sensorineural hearing loss in both children and adults. Moreover, conducting these studies in young children is very simple (they only require a sample of blood or saliva). Therefore, genetic counselling should be a fundamental pillar in the diagnosis of sensorineural hearing loss.

**Usefulness of Genetic Diagnosis**

Identifying the presence of a genetic predisposition within a family has multiple advantages for both healthy individuals and those who have already manifested the disease. It is very important to note that different families and individuals expect to obtain different information from genetic counselling. In some cases their interest is mainly focused on reproductive and family planning issues. In others, their interest is to know the cause of their deafness and its medical and social implications. Therefore, objectives should always be oriented towards the concerns of patients and their families.

The benefits for family members may include the possibility of detecting carriers who are currently asymptomatic, assessing the risk of transmission to offspring and avoiding uncertainty, anxiety and unnecessary testing of non-carriers.

The benefits for patients can be classified into five main areas: prevention, diagnosis, prognosis, treatment and reproduction.

- Prevention: preventive measures are effective in hereditary hearing loss conditioned by environmental factors, such as mutations conferring sensitivity to aminoglycosides or cisplatin toxicity. Often, deafness may be the first manifestation of a more complex syndrome. Therefore, when the mutation identified may cause syndromic manifestations, it is essential to contemplate the potential involvement of other organs, even if this is not present at the time of diagnosis (loss of vision, cardiac conduction defects, kidney failure, etc.).

- Diagnosis: knowing the cause of deafness can often help patients or their parents to emotionally assimilate diagnosis, accelerating their involvement in rehabilitation treatment. In addition, a genetic diagnosis can prevent unnecessary testing (CT, MRI, neurophysiological studies, etc.).

- Prognosis: it is often possible to predict the evolution of deafness, establishing a prognosis based on the genotype of each patient. In this case, genetic studies condition the monitoring protocol, hearing rehabilitation plan and educational decisions (rapidly progressive hearing loss affecting all frequencies versus stable hearing loss involving only a specific range of frequencies).

- Treatment: knowing the cause of deafness may be useful when making treatment decisions. For example, patients with hearing loss secondary to mutations in the *GJB2* and *GJB6* genes obtain more benefit from cochlear implants than patients with hereditary hearing loss without mutations in these genes, with the results of implantation usually being excellent. Patients with hereditary hearing loss with predominance in high and middle tones who are stable over time (for example, certain mutations in the TECTA gene) may be good candidates for shorter cochlear implants, supported by hearing aids. On the other hand, hearing loss and vestibular symptoms caused by mutations in the *COCH* gene, have occasionally been associated with superior semicircular canal dehiscence, in which case surgical improvement of symptoms is a possibility. On the other hand, the correct treatment of neuropathies requires knowledge of their aetiology, discriminating between different genetic and environmental causes. Thus, for example, auditory neuropathy secondary to mutations in the *OTOF* gene does not affect auditory nerve function (see specific section). Therefore, cochlear implants usually provide a good performance in these patients. Specific mutations in the *PJVK* gene can induce primary neural dysfunction and, therefore, compromise the results of potential cochlear implantation in patients with a similar audiometric profile to that observable with mutations in the *OTOF* gene.

- Reproduction: after identifying a gene and its inheritance pattern it is possible to accurately determine the probability of having deaf children. Thus, for example, two parents affected by AR inheritance hearing loss will have deaf descendants in 100% of cases if their mutations are in the same gene. Conversely, if the mutations affect different genes, none of their children will inherit the disorder. Identification of the mutation responsible will enable discussion of the available reproductive options, as well as the associated legal and ethical considerations (preimplantation genetic diagnosis, prenatal genetic diagnosis). It may also be possible to determine the presence or absence of the mutation in the newborn, thus speeding the diagnostic and therapeutic processes. In those cases where it is not possible to identify the gene responsible or to establish an inheritance pattern, it may be possible to use empirical percentages in the counselling process. Thus, for example, a priori, when a couple with normal hearing and no family history of hearing loss have a deaf child, their chance of having another deaf child is 18%. The probability that a couple formed by one deaf individual and one with normal hearing have a deaf child is 10%. If a genetic study has been performed and has found no pathogenic mutations these figures may be modified, depending on the genes analysed.

In addition to the usefulness for patients and their families, we must also bear in mind the importance of genetic studies to advance the knowledge of inherited hearing disorders. Including patients in homogeneous aetiological groups helps to further the genotype-phenotype correlation and helps to establish the efficacy of various therapeutic measures in these different groups. Moreover, knowing the prevalence of different mutations within our population increases the efficiency of genetic studies. In the future, a deeper understanding of the pathophysiological mechanism responsible for a particular type of hearing loss could enable specific therapies directed against specific molecular alterations.
Table 1 Genes Involved in Non-syndromic Genetic Hearing Loss With Autosomal Recessive Inheritance.

<table>
<thead>
<tr>
<th>Locus</th>
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<th>Protein</th>
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<td>DFNB85</td>
<td>GJB3</td>
<td>Connexin 31&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> These genes can also cause syndromic hearing loss.

Non-syndromic Genetic Hearing Loss: Genes Most Frequently Involved

Most of the genes responsible for non-syndromic genetic hearing loss encode proteins involved in the development, structure and function of the cochlea. Tables 1–3 list the over 50 genes identified so far.<sup>5</sup> We should note that, due to the allelic and genetic heterogeneity described previously, a single gene may appear in more than one table. Moreover, some of these genes are also responsible for syndromic hearing loss.

As a general rule, we can say that the majority of recessive hearing losses (DFNB) cause a moderate to deep pre-lingual deafness, which is stable over time. By contrast, dominant hearing losses (DFNA) are usually post-lingual and progressive. Those linked to the X chromosome (DFNX) may be both pre-lingual and post-lingual. However, there are numerous exceptions to this rule. Mutations in genes such as TMPRSS3 (DFNB8/DFNB10), MYO3A (DFNB30), PJVK (DFNB59), LOXHD1 (DFNB77) or TPRN (DFNB79) may lead to progressive hearing loss with AR inheritance.<sup>42,43</sup> In these cases, when hearing loss begins in the third or fourth decade of life it could be mistaken for sporadic or environmentally caused hearing loss, given the progressive nature and inheritance pattern. By contrast, carriers of certain heterozygous mutations (AD inheritance) may develop early-onset profound hearing loss (for example, mutations in GJB6-DFNA3- or TECTA-DFNA8/DFNA12-).

Table 2 Genes Involved in Non-syndromic Genetic Hearing Loss With Autosomal Dominant Inheritance.

<table>
<thead>
<tr>
<th>Locus</th>
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<th>Protein</th>
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<td>CRYM</td>
<td>μ-crystallin</td>
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<sup>a</sup> These genes may also cause syndromic hearing loss.
Hereditary Hearing Loss: Genetic Counselling

Table 3  Genes Involved in Non-syndromic Genetic Hearing Loss With X-linked or Mitochondrial Inheritance.

<table>
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Mitochondrial Inheritance

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<td>MTTS1</td>
<td>Mitochondrial serine transfer 1 RNA</td>
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</table>

a These genes may also cause syndromic hearing loss.

Next, we will carry out a brief description of those genes whose mutations are identified most frequently in our environment.

Connexins

Connexins are a family of proteins responsible for the formation of intercellular channels. When these channels are grouped they originate so-called gap junctions. These channels enable the gradient-wise flow of ions and metabolites between adjacent cells. Each channel is formed by two hemichannels or connexons. In turn, each connexon is composed of six connexin molecules. Gap junctions in the cochlea are formed by four types of connexins: connexin (Cx) 26 (GJB2 gene), Cx30 (GJB6 gene), Cx31 (GJB3 gene) and Cx43 (GJA1 gene). Their correct function is necessary for the maintenance of ionic and metabolic cochlear homeostasis. Their alteration would result in the onset of hearing loss through various mechanisms (altered potassium flows, impairment of the endothelial barrier in the stria vascularis, etc.).

Mutations in the GJB2, GJB6 and GJB3 genes together represent the most common cause of hereditary non-syndromic hearing loss in our environment. Mutations in the GJA1 gene cause different syndromes which are associated with conductive hearing loss (columodontidigital dysplasia, Hallermann-Streiff syndrome or hypoplastic left heart syndrome).

GJB2

Its mutations are the most common cause of non-syndromic hearing loss with AR inheritance (DFNB1A). To date, over 100 pathogenic mutations have been identified in this gene. However, the 35delG mutation is responsible for most mutant alleles in populations of European origin. The high prevalence of these mutations (up to 3% of the Caucasian population can be carriers) and the existence of a recurrent mutation have made the study of this gene part of the routine molecular diagnosis of most laboratories.

Patients carrying a homozygous mutation in GJB2 usually suffer severe early-onset hearing loss. However, although the phenotype is usually homogeneous, the GJB2 gene is a good example of allelic heterogeneity. On the one hand, it has been possible to observe every degree of hearing impairment (moderate to profound), and even asymmetric hearing loss, in patients with homoygous mutations.

On the other hand, mutations in GJB2 may be responsible for both non-syndromic hearing loss with autosomal dominant inheritance (DFNA3A) and for different syndromes with more or less severe phenotypes. The latter may range from changes in skin pigmentation to mutilating keratoderma (Vohwinkel syndrome). There have been attempts at establishing a genotype-phenotype relationship in which the severity of the deficit is correlated with the specific type of mutation, obtaining conclusive results in studies with a sufficient number of patients.

Among populations with a European origin it is not uncommon to encounter patients with one heterozygous mutation in GJB2 and another, also heterozygous, in GJB6. These patients are known as compound heterozygotes.

GJB6

Mutations in this gene are the second cause of pre-lingual hereditary hearing loss in the Spanish population, following the 35delG mutation of GJB2. They usually cause non-syndromic hearing loss with AR inheritance (DFB1B), with patients being either homozygotes or compound heterozygotes (one mutation in GJB2 and another in GJB6). However, this gene is also responsible for AD non-syndromic (DFNA3B) or syndromic (Clouston syndrome) hearing losses. Overall, mutations in GJB2 and GJB6 are responsible for approximately 50% of non-syndromic hearing losses with AR inheritance (DFNB1).

GJB3

As is also the case with other cochlear connexins, mutations in the GJB3 gene can result in non-syndromic hearing loss with both AR and AD inheritance (DFNA2B) or in syndromic cases with cutaneous or neurological manifestations. As occurred with GJB6 there have been recent reports of compound heterozygous patients, carriers of mutations in both GJB2 and GJB3.

OTOF

The OTOF gene encodes the protein otoferlin, which is necessary for the proper functioning of the synapses between inner ciliated cells and underlying neurons (type I fibres of the cochlear nerve). Their mutations usually result in AR non-syndromic hearing loss, with a specific and homogeneous phenotype (DFNB9). Homozygous individuals present pre-lingual profound hearing loss with preservation of otocoustic emissions and absence of brainstem auditory evoked potentials (auditory neuropathy). Since the auditory nerve works correctly, cochlear implants in patients with mutations in the OTOF gene offer a similar performance to that obtained in other cochlear hearing losses. A recurrent mutation in this gene (Q829X) has been identified in Spain and among Hispanic populations. In Spain, this mutation is the third leading cause of genetic pre-lingual deafness, after connexin mutations. It is worth noting that certain mutations of this gene in heterozygosis may give rise to an intermittent hearing loss, precipitated by increased temperature (for example, fever episodes).
**COCH**
Mutations in this gene are the most common cause of AD non-syndromic hearing loss (DFNA9). Its heterozygous mutations cause progressive late-onset hearing loss, with predominance in high frequencies, usually associated with vestibular dysfunction.\(^\text{35}\) When they appear, vestibular symptoms are similar to those of Menière’s disease (vertigo, tinnitus, aural fullness and hearing loss) and may precede auditory deterioration by up to 10 years.\(^\text{60}\) Therefore, its analysis should be considered in patients with suspected Menière’s disease and a family history, especially if hearing loss preferentially affects high tones.

**WFS1**
Heterozygous mutations of the WFS1 gene may be asymptomatic or cause non-syndromic late-onset deafness, predominantly in low tones (DFNA6/DFNA14/DFNA38), prelingual hearing loss or even Wolfram syndrome.\(^\text{12}\) Wolfram syndrome (diabetes insipidus, diabetes mellitus, optic atrophy and deafness [DIDMOAD])\(^\text{61}\) is usually due to a homozygous mutation of WFS1. This syndrome is an example of genetic heterogeneity (it can also be caused by mutations in the CISO2 gene\(^\text{62,63}\)) with variable expressivity (manifestations may range from moderate hearing loss with predominance in high tones and type 2 diabetes to severe cases, with significant neurological deficits).\(^\text{64,65}\) A recurrent mutation (425ins16) has been identified among the Spanish population, which is present in over 50% of families with Wolfram syndrome.\(^\text{66}\)

**MIRN96**
This gene does not encode a protein but a small fragment of RNA with a regulatory function (micro-RNA) expressed mainly in cochlear ciliated cells. Its mutations cause the development of hereditary non-syndromic hearing loss (DFNA50). These mutations are of particular interest in our environment, because they were initially described in the Spanish population and because they represent the first mutation responsible for deafness identified in microRNA.\(^\text{67,68}\)

**POU3F4**
This gene is located on the X chromosome and its mutations cause progressive early-onset hearing loss (DFNX2), which can be sensorineural or mixed (it may appear with stapes fixation). During stapedectomy, these patients are at risk of sudden and massive perilymph leakage (gusher). CT scans can identify a dilatation of the internal auditory canal and dehiscence of its lateral wall.\(^\text{69,70}\)

**Mitochondrial Genes**
Generally, mutations in mitochondrial genes cause complex syndromes. However, certain mutations in genes such as *MTRNR1, MTTS1* or *MTTL1* may present hearing loss as their only symptom. In addition to their characteristic inheritance pattern, these disorders display high variability in terms of penetrance and severity. The most characteristic example is the 1555G→A mutation in the *MTRNR1 (12sRNA)* gene. This mutation is considered as the most common cause of post-lingual hearing loss in the Spanish population.\(^\text{28,30}\) In some individuals, hearing loss is triggered as a result of aminoglycoside antibiotics, while in others hearing loss appears spontaneously.\(^\text{6,71}\)

**Syndromic Genetic Hearing Loss: Most Common Syndromes**
As discussed, hearing loss is an identifiable symptom in over 400 different syndromes.\(^\text{6}\) However, in most syndromes hearing loss is mild or may even be absent. An adequate clinical evaluation within a multidisciplinary environment is essential to rule out syndromic hearing loss, since this diagnosis may have great relevance for the clinical management of patients (visual alterations, heart disease, kidney disease, etc.). As with non-syndromic hearing loss, in most syndromes the cause of deafness is located in the organ of Corti or the stria vascularis. However, in some cases dysfunction also affects neurons in the spiral ganglion, causing sensory and neural hearing loss, with subsequent diagnostic and therapeutic implications. A detailed analysis of the different syndromes is beyond the scope of this review, but the most common syndromes, as well as some of their most relevant clinical data, are described below, in order of frequency.

**Autosomal Recessive Inheritance**
- Usher syndrome: pre-lingual sensorineural hearing loss and retinitis pigmentosa. The latter usually appears during the second decade of life. Patients are classified into three groups depending on the type of hearing loss, the presence or absence of vestibular involvement and the age of onset of retinitis. A total of nine genes responsible for this syndrome have been identified (*MYO7A, USH1C, CDH23, PCDH15, SANS, USH2A, VLGR1, WHRN, and USH3*).\(^\text{7}\)
- Pendred syndrome: sensory hearing loss, usually pre-lingual. The syndrome is characterised by the presence of goitre, which can develop either during puberty or in adulthood. Thyroid function is normal in over half of cases.\(^\text{7}\) Vestibular dysfunction and bone abnormalities in the inner ear are often present (Mondini dysplasia or dilated vestibular aqueduct). In 50% of cases it is possible to identify a mutation in the *SCL26A4* gene. The study of this gene would be indicated in patients with progressive hearing loss and radiological abnormalities of the bony labyrinth, regardless of the presence or absence of goitre (this gene is also responsible for DFNB4 non-syndromic hearing loss).\(^\text{22,23}\) Less frequently, Pendred syndrome may be caused by mutations in the *FOX1* gene.\(^\text{7}\)
- Jervell and Lange-Nielsen syndrome: pre-lingual sensorineural hearing loss and prolonged QT interval. Affected patients are at risk for sudden death. Two responsible genes have been identified (*KCNE1* and *KCNO1*).\(^\text{7}\)

**Autosomal Dominant Inheritance**
- Waardenburg syndrome: sensorineural hearing loss of varying severity (mild or profound), unilateral or bilateral.\(^\text{10}\) Associated abnormalities in skin, hair (characteristic white forelock) and eye (heterochromia iridis) pigmentation. Four types have been described and six
genes have been identified (PAHX3, MITF, SNAI2, EDNRB, EDN3, and SOX10).16
- Branchio-oto-renal syndrome: highly variable expressivity. Perceptive hearing loss, conductive or mixed, which may be associated with branchial cysts or fistulae, anomalies of the external ear canal and/or the ear, and kidney malformations. A total of three genes have been identified to date (EYA1, SIX1, and SIX5).17,18

X-linked Inheritance
- Alport syndrome: post-lingual progressive hearing loss, associated with progressive glomerulonephritis. It often presents ocular abnormalities, with the most characteristic being anterior lenticonus. In 85% of cases, inheritance is linked to the X chromosome (COL4A5 gene), but there have also been reports of autosomal forms (COL4A3 and COL4A4 genes), either AR, or occasionally AD.79

Mitochondrial Inheritance
Generally, mitochondrial disorders are preferentially manifested in tissues with a high energy demand. Thus, mutations in the mitochondrial genome can cause many different syndromes, ranging from severe neuromuscular disorders (MERRF, MELAS, LHON, etc.) to milder and more common symptoms, such as diabetes mellitus type 2. Involvement of the auditory pathway is frequent in these syndromes with a mitochondrial origin. The common factor is the characteristic inheritance pattern (affecting both genders equally, only transmitted by the mother and affecting 100% of the offspring of an affected female). However, when evaluating a genealogy it is important to bear in mind the incomplete penetrance and variable expressivity of these syndromes. Among the genes identified so far we highlight MTTL1, MTT51, MTND1, MTND4, MTND5, MTND6, and MTND4L.6

Molecular Studies Available in Common Clinical Practice
When a specific syndrome has been clinically identified, it is possible to direct the genetic study towards the genes responsible. If this is not feasible (family refusal, unavailability of adequate laboratory, technically complex genes, etc.) and the syndrome is clinically well defined, advice can be given assuming that the patient carries the mutation responsible for the syndrome. Conducting a karyotype may be indicated in the presence of dysmorphic features or developmental delays. It is important to remember that sometimes the signs which define the syndrome may manifest years after the onset of deafness, making it then necessary to reassess the initial diagnosis of non-syndromic hearing loss.

In case of non-syndromic sensorineural hearing loss, genetic tests are the diagnostic test of choice.9,14,16,26,27 In order for genetic studies to become part of routine clinical practice it is important that their results can be obtained within a reasonable time. Faster results will lead to less anxiety for patients and/or their parents, and will enable physicians to adapt therapeutic and rehabilitative measures to the aetiological diagnosis. As mentioned, in some patients the presence of certain clinical or radiological signs and the inheritance pattern identified in the family may direct the molecular study towards specific genes (for example, SLC26A4, POU3F4, OTOF, or MTRNR1). In other cases it may be possible to select the genes to be studied through the audiometric profile (for example, mutations in the WFS1 gene are detected in 75% of families with AD hearing loss, initially affecting only deep tones69). However, in most cases it is not feasible to establish a good genotype-phenotype correlation, thus making it necessary to approach the study in an empirical manner. In addition, we must bear in mind that, depending on the particular mutation, the existence of genetic modifiers and environmental variables, it may possible for mutations in the same gene to manifest as AD or AR. It is also necessary to consider the possibility of the existence of a homozygous mutation in families with an apparently AD inheritance pattern (two or more generations affected). This may occur due to the high prevalence in our environment of carriers of mutations in the GJB2 gene. The possibility of hearing loss appearing in double heterozygotes (carriers of mutations in GJB2 and GJB6 or GJB2 and GJB3) also contributes to this pseudodominant inheritance pattern.

Given the great heterogeneity of hereditary hearing loss and the prohibitive cost of studying all the genes identified so far (over 50), any molecular approach employing conventional sequencing techniques must be limited to the study of those genes most frequently mutated in the population and ethnic group to which the family belongs.81,82 Therefore, the effectiveness of any genetic testing protocol will be conditioned by the knowledge of the target population (most frequent genes and mutations).83 In our environment, most cases of hereditary non-syndromic hearing loss are caused by mutations in a few genes (for example, GJB2, GJB6, OTOF, MTRNR1).17,50 A basic study must necessarily include the GJB2 and GJB6 genes.7 Some laboratories offer the possibility of analysing other genes among their routine tests, either sequentially or in a single experiment, employing different techniques.84-86 The extreme genetic heterogeneity of hereditary hearing loss and variability in the aetiological distribution among different populations make sequential approaches inadequate in terms of efficiency and cost.81,87,88 By contrast, simultaneous studies are faster and are usually easier to interpret (variants of uncertain significance, modulation of the effect of mitochondrial mutations by somatic mutations, double heterozygotes, etc.).51,87,89 One way to solve the problem of genetic heterogeneity is through the use of gene chips. These platforms can detect predetermined point mutations within a large number of genes in a single experiment.84 Since they only detect those mutations included in the chip, their efficacy is influenced by the genetic knowledge of the population in which they are employed. On the other hand, sequencing techniques do not require knowing the mutation being sought a priori. When interpreting the results of a genetic study we must bear in mind that conventional sequencing techniques are unable to identify the presence of large deletions or duplications. This requires the use of complementary techniques such as MLPA (multiplex ligation-dependent probe amplification). Both deletions and duplications of previously mentioned genes may cause hereditary hearing loss.51,90-92
When faced with a negative result we must remember that this only indicates an inability to detect a mutation in those genes analysed, but does not exclude the possibility that the cause of deafness is genetic. It is essential for this information to be correctly transmitted to patients and/or their families (for example, this would not eliminate the risk of bearing deaf children in the future).

Future Perspective

Currently, second-generation sequencing technologies enable the human genome to be sequenced in a single experiment within a few weeks. Their potential has been demonstrated through their application in the discovery of the genetic cause of several hereditary diseases.\(^1\)\(^3\)\(^-\)\(^5\) Recently, this technology has been used to simultaneously sequence 54 genes associated with non-syndromic hearing loss in eight deaf individuals, and succeeded in identifying the gene responsible in seven patients.\(^6\) At present, methodological limitations make the analysis of the sequences obtained even more complex, while the need to validate the results obtained increases the costs of the process.\(^6\) However, in the future, it will be feasible to analyse the complete genome of an individual at a cost similar to that of currently sequencing a single gene with several coding exons (for example, MYO7A or CDH23) through first-generation technology (Sanger sequencing\(^7\)). The future use in everyday clinical practice of second- and third-generation sequencing is expected to revolutionise clinical practice and, of course, will radically influence the management of hereditary diseases.

Conflict of Interests

The authors have no conflicts of interest to declare.

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Hereditary Hearing Loss: Genetic Counselling


