Aetiological Diagnosis of Child Deafness: CODEPEH Recommendations

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Abstract Important progress in the fields of molecular genetics (principally) and diagnostic imaging, together with the lack of a consensus protocol for guiding the diagnostic process after confirming deafness by neonatal screening, have led to this new work document drafted by the Spanish Commission for the Early Detection of Child Deafness (Spanish acronym: CODEPEH). This 2015 Recommendations Document, which is based on the most recent scientific evidence, provides guidance to professionals to support them in making decisions regarding aetiological diagnosis. Such diagnosis should be performed without delay and without impeding early intervention. Early identification of the causes of deafness offers many advantages: it prevents unnecessary trouble for the families, reduces health system expenses caused by performing different tests, and provides prognostic information that may guide therapeutic actions.
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PALABRAS CLAVE
Sordera; Diagnóstico; Etiología; Genética; Hipoacusia; Atención temprana

Diagnóstico etiológico de la sordera infantil: recomendaciones de la CODEPEH

Resumen El importante avance en el campo de la genética molecular, fundamentalmente, así como en el diagnóstico por imagen, junto a la ausencia de un protocolo consensuado que oriente el proceso diagnóstico una vez confirmada la presencia de una sordera tras el cribado neonatal, motivan este nuevo trabajo de la Comisión para la Detección Precoz de la Hipoacusia Infantil (CODEPEH). El Documento de Recomendaciones sobre el diagnóstico etiológico de la sordera,

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Introduction

The importance of diagnosing hearing loss early has been well acknowledged for decades, from both a scientific and empirical perspective. Many countries have now implemented universal new-born hearing screening programmes in line with the recommendations of the Joint Committee on Infant Hearing, who advocate that detection should not be delayed beyond the first month of life and it is possible to confirm a diagnosis in the third month in order to ensure that infants receive the correct treatment before the age of 6 months, given that the main objective is for children to acquire spoken language and achieve maximum development with a hearing deficit at a personal, cognitive, educational and social level. Within this process, the need for an aetiological diagnosis protocol has become one of the main foci of attention of practitioners in this area. The limit between genetic and environmental hearing loss has not been clearly defined. Despite the fact that it is calculated that 60% of early onset deafness is genetic and 40% environmental, the presence of the latter does not exclude a genetic predisposition. In a study undertaken in neonates with confirmed hearing loss an aetiological factor was found in almost half the cases, and of these, more than 60% were of genetic origin, 20.8% due to perinatal problems and 18.8% to congenital infection by cytomegalovirus.

Early identification of the cause of hearing loss has several advantages. It prevents costly and unnecessary tests, reduces the stress of parents and children, enables genetic counselling and provides us information on the prognosis, so that we can identify and even anticipate potential coexisting medical problems. All of which serves as a guide for successful therapeutic action. At this time, given the practice of undertaking an extensive battery of expensive tests simultaneously in all children with hearing loss, an algorithm is required to guide practitioners towards an efficient aetiological diagnosis, which should be reached in a manner that does not hinder or delay early intervention. To that end, CODEPEH consider it necessary to formulate recommendations, given the significant advances achieved in the field of molecular genetics, essentially, and in diagnostic imaging. These recommendations are based on the most recent scientific evidence and aim to bring order to a process where there are no agreed protocols resulting in cases that are left undiagnosed, or the indiscriminate use of numerous tests causing unnecessary discomfort to the children and their parents, as well as unjustified health-care costs.

In short, with their new recommendation document, CODEPEH seek to offer support guidelines to professionals in decision-making during the process of aetiological diagnosis, to the extent possible in order to avoid the variability in clinical action which has been observed and documented in other countries.

Diagnostic Sequence

Correct aetiological guidance requires comprehensive collection of family and personal histories, including risk factors, a detailed physical examination and, when necessary and relevant to these paragraphs, the appropriate complementary studies.

Anamnesis and Physical Examination

In order to gather data on the patient’s family history it is a good idea to make a family tree, bearing in mind that several premises must be met for this to be valid. An attempt should be made to collect data from 3 generations, with special emphasis on first-degree relatives (from whom otological and audiological examinations should be collected); looking at factors such as family tree dynamics, by periodically reassessing them, as well as false paternities, adoptions, assisted reproduction techniques (egg/sperm donation) and/or the appearance of de novo mutations, and ask questions about inheritance pattern, consanguinity, ethnicity and country of origin.

Both the mother and the father’s health data should be recorded in the clinical history. It should include information on the pregnancy, delivery and the neonatal period. Exposure to medication, drugs and/or toxins during pregnancy should be enquired about. It should not be forgotten that pre and perinatal infections (Table 1), which can be diagnosed in the mother, foetus and the neonate, are some of the most common causes of deafness. We detail some of these infections below that are studied and followed-up during pregnancy, so that hearing loss can be suspected at birth enabling early diagnosis:

1. **Toxoplasmosis.** Infection is asymptomatic in most pregnant women. Definitive diagnosis of maternal infection is seroconversion of immunoglobulin Ig G during gestation. Polymerase chain reaction (PCR) of the suspected germ in amniotic fluid is determined to diagnose foetal infection.
<table>
<thead>
<tr>
<th>Congenital infection virus</th>
<th>Type of deafness</th>
<th>Laterality</th>
<th>Level</th>
<th>Incidence</th>
<th>Prevention</th>
<th>Treatment</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td>Sensorineural</td>
<td>Bilateral</td>
<td>Severe</td>
<td>6%-23% asymptomatic; 22%-65% symptomatic</td>
<td>No</td>
<td>Valganciclovir</td>
<td>With treatment</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis</td>
<td>Sensorineural</td>
<td>Bilateral</td>
<td>Severe</td>
<td>7.4%</td>
<td>Isolation</td>
<td>Ribavin, Favipiravir</td>
<td>No</td>
</tr>
<tr>
<td>Rubella</td>
<td>Sensorineural</td>
<td>Bilateral</td>
<td>Moderate-severe</td>
<td>12%-19%</td>
<td>Vaccination</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>Sensorineural</td>
<td>Bilateral</td>
<td>Moderate-severe</td>
<td>27.5%-33.5%</td>
<td>Treatment post-exposure</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Herpes simple</td>
<td>Conductive</td>
<td>Bilateral</td>
<td>Moderate-severe</td>
<td>&lt;33%</td>
<td>No</td>
<td>Acyclovir</td>
<td>No</td>
</tr>
<tr>
<td>Acquired infection</td>
<td>Type of deafness</td>
<td>Laterality</td>
<td>Level</td>
<td>Incidence</td>
<td>Prevention</td>
<td>Treatment</td>
<td>Recovery</td>
</tr>
<tr>
<td>Measles</td>
<td>Sensorineural</td>
<td>Bilateral</td>
<td>Severe</td>
<td>0.1%-3.4%</td>
<td>Vaccination, Ig</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Varicella zoster</td>
<td>Sensorineural</td>
<td>Unilateral</td>
<td>Mild-moderate</td>
<td>7%-85%</td>
<td>Vaccination</td>
<td>Acyclovir</td>
<td>Variable</td>
</tr>
<tr>
<td>Parotiditis</td>
<td>Sensorineural</td>
<td>Unilateral</td>
<td>Variable</td>
<td>&lt;4%</td>
<td>Vaccination</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>West Nile virus</td>
<td>Sensorineural</td>
<td>Bilateral</td>
<td>Moderate-severe</td>
<td>Very rare</td>
<td>Vaccination</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Modified from Cohen et al.\textsuperscript{18}
2. Syphilis. Diagnosis is serological, by nontreponemal and treponemal tests. It is possible to detect *Treponema pallidum* in the amniotic fluid for prenatal diagnosis of congenital infection.

3. Rubella. The diagnosis of maternal infection involves proving an increase in IgG titre 4 times greater than the initial level, and IgM specific to rubella, either by identifying the virus in urine or nasopharyngeal secretions by PCR. The virus culture is of low sensitivity. Prenatal diagnosis is made by detecting IgM in foetal blood (obtained after week 22), direct detection of the virus in the chorionic villi or PCR in amniotic fluid.

4. HIV. This can be detected by fast techniques such as chemo luminescence to detect the HIV 1–2 antigen–antibody, and positive or borderline results are confirmed with Western Blot in the neonate. In the event of a positive result the virus must be quantified by blood PCR.

There are other pre-perinatal infections with a high incidence of deafness which are not systematically screened, and therefore their suspicion depends on the symptoms presented by the foetus or the neonate. This is the case with the following viruses:

1. Cytomegalovirus (CMV). This is currently the most frequent cause of congenital infection and one of the causes of deafness which occasionally is postnatal and progressive. CMV causes deafness in 10%–20% of children with proven hearing loss, although in some studies it is 30%.

   Most neonates are asymptomatic at birth. Approximately 10%–15% of asymptomatic neonates will go on to develop hearing loss. Some of these will have altered results in the neonatal hearing screening process. Up to 75% of children with congenital infection were identified in several studies thanks to alterations in the hearing screening process. Nine percent presented deafness of later onset (they are not therefore candidates for diagnosis within a neonatal screening process), it being progressive in 20% of cases through childhood. In symptomatic cases, 30%–50% will have deafness that might be detected at birth, but in 18%–30% it will present later, and can be progressive in up to 63% of cases, through the first 6 years of life, and can become profound in 78%. The risk of vertical transmission is much greater in primary infection than in recurrent infections (32% vs 1.4%), as is the severity of symptoms. CMV study is indicated in babies with proven hearing loss. Similarly it should be considered for use in asymptomatic cases that present altered final results in neonatal hearing screening, and are referred to the ENT specialist for confirmation. The deadline for diagnosing congenital infection with certainty is 2–3 weeks postnatally. Within this period, a PCR of the germ should be taken from urine, saliva or blood. If the neonate is older than 2.3 weeks, this PCR will not be decisive, therefore PCR on blotting paper should be used in the test for metabolopathies to confirm the virus.

2. Herpes virus. This is diagnosed by viral culture and determining PCR of vesicles, conjunctiva, oropharynx, blood and CSF. Serology is of little value, although persistent IgG for more than 6–12 months confirms neonatal infection. According to some studies, herpes can cause deafness in the same way as CMV, although this seems to be rarer.

3. Neonatal varicella. Diagnosis is clinical, but serological confirmation is recommended, IgG and IgM, with 2 samples over a 15-day interval. Specific PCR can also be detected in skin lesions. It rarely causes deafness.

4. Other germs. It should be remembered that the parotiditis virus, Nile virus and many others can cause deafness in children.

In addition to the above, other backgrounds that entail a risk should be taken into account, such as cranio-encephalic trauma, exposure to ototoxic or chemotherapy medication, admission to intensive care (assisted ventilation, extracorporeal membrane ventilation, hyperbilirubinaemia with exanguinotransfusion, great prematurity, perinatal hypoxia), other perinatal infections, including bacterial or viral meningitis, neurodegenerative diseases, craniofacial anomalies and persistent otitis.

In relation classifying hearing loss as syndromic or non-syndromic, there are several signs on physical examination that should be recognised which might indicate some type of syndrome, since it is estimated that up to 30% of hearing loss of genetic cause are syndromic. Therefore, physical examination should focus on dysmorphic features and other clinical signs, such as the following: the patient’s height, body habitus, skin colour, hair and skin lesions, and craniofacial morphology. The size and morphology of the pinna and area of implantation should be examined. It is also important to establish whether there are preauricular tags or pits, as well as aural atresia. It is important to highlight the palpebral fissure angle, intercanthal distance, morphology and colour of the iris and cornea, visual acuity, not forgetting the oculomotor muscles. Hair lip or cleft palate are associated with hearing loss. Many syndromes present with facial anomalies and hearing loss, therefore data should be gathered on facial morphology, bone and/or muscle development of the face, and nasal morphology. Data should be gathered on the morphology and length of the neck, its mobility and the existence of masses. Furthermore, the morphology and size of the limbs are important.

**Complementary Tests**

**Genetic Tests**

Most congenital sensorineural hearing loss is non-syndromic and of genetic aetiology, therefore genetic tests have been demonstrated to provide the highest diagnostic yield. Aetiological diagnosis of genetic hearing loss is very complex and there are no standardised protocols. Traditionally, genetic diagnosis has been based on Sanger’s sequencing, developed in 1975 and based on PCR. This very sensitive and specific test is the benchmark for analysing one or a few genes, but its costs and time render it impractical for simultaneously sequencing dozens of genes. The technological advances of recent years in the field of genomic sequencing have radically changed genetic diagnosis of polygenic hereditary diseases, such as hearing loss. These advances, unprecedented throughout the history of molecular biology, now enable that which would have been considered utopian less than 10 years ago:
the sequencing of as many genes as required (from dozens to the complete genome) in times and at costs compatible with routine medical care.\textsuperscript{14} This set of technologies termed next generation sequencing (NGS), enable 3 approaches to the diagnosis of hereditary diseases\textsuperscript{15,16,17}: (a) complete genome (sequencing of the entire genome); (b) exome (sequencing of the regions of the genome that code the necessary information for protein synthesis [exons]), and (c) gene panel (sequencing of a set of genes associated with a specific disease) At present, in clinical practice, gene panels are considered the most appropriate method for the genetic diagnosis of deafness.\textsuperscript{5,17} The expected diagnostic yield from these panels is around 50\%.\textsuperscript{23,24} This figure is very variable, ranging from 13\% to 100\%, this difference is influenced by the methodology used and population analysed.\textsuperscript{25,26} It is likely that in coming years the diagnostic rates will increase as new genes are associated with the development of hereditary hearing loss.\textsuperscript{18} When it is not possible to reach a diagnosis using a panel and the suspicion of an underlying genetic cause remains, exomes are an appropriate tool to identify new genes involved in hearing loss.\textsuperscript{27,28} As yet exomes should be reserved for research since they are more expensive than panels, are more difficult to interpret and the results take more time to process.\textsuperscript{23,25} Another disadvantage with exomes is that, as a part of analysis, gene variants can be detected that are involved in diseases other than deafness (e.g., neurodegenerative diseases, hereditary heart disease, etc.), creating difficulties when it comes to genetic counselling these patients.\textsuperscript{23}

If the histories, examination and studies requested do not conclude that the hearing loss is acquired, or there are no clinical indications for this to be suspected, an attempt should be made to confirm genetic aetiology. Therefore it is necessary to refer the patient for genetic counselling, in accordance with the algorithm in \textbf{Fig. 1}.\textsuperscript{1,2,3,21} In Spain, the law establishes the need for a process of genetic counselling before and after all genetic testing, and the need for specific informed consent. In this consultation, which must form part of the multidisciplinary team caring for the patient with hearing loss,\textsuperscript{1} the hearing loss must be characterised as thoroughly as possible. In cases where clinical examination suggests that a particular gene or set of genes might be responsible for the phenotype, it is possible to request a targeted genetic study (for example, mitochondrial mutations with a compatible inheritance pattern and a history of aminoglycoside ototoxicity). On occasion, when a syndrome that might be caused by several genes is suspected (Usher’s syndrome, for example), it might be more effective, in terms of time and costs, to request targeted NGS of a panel that includes the genes of interest. In the majority of cases it will not be possible to identify a candidate gene from the phenotype. Fortunately, the current advances in sequencing techniques, in the interpretation of bioinformatics and the reduction of the costs of the different steps, makes it possible to reach a genetic diagnosis rapidly, regardless of the phenotype, and without the need for additional confirmatory studies.\textsuperscript{24} In this regard, with a view to minimising the costs of the process, the first step recommended is to study the presence of GJB2 mutations and GJB6 deletions, given their high prevalence in our environment.\textsuperscript{18,35} If it is not possible to identify the cause of deafness after analysing these genes, the next step should be the sequencing of an appropriately selected panel of genes by NGS.\textsuperscript{5,27} When the panel is selected, attention must be paid to the genes included, their sensitivity and specificity, and their capacity for detecting variations in the number of copies. It should never be forgotten that a negative result only indicates that a mutation in the genes analysed has not been detected, but it does not rule out the possibility of a genetic cause for the hearing loss. It is essential that this information is appropriately transferred to the patient and/or their family (for example, it would not eliminate the risk of their having other deaf children). Periodic follow-ups should also be planned (every 3 years, for example) with the genetic counselling specialist. Thus it will be possible to identify syndromic traits of new onset that might not have been evident at the time of initial evaluation. These follow-ups should also offer the patient the possibility of new genetic tests or to reinterpret those already undertaken, as our knowledge advances.

**Imaging Tests**

Radiological assessment by computerised tomography (CT) and/or magnetic resonance (MR) is important in studying the aetiology of neonatal hearing loss.\textsuperscript{36} Both scans provide different features for evaluating the different pathologic anatomic alterations in the external, middle and internal ear, and the central auditory pathways. The temporal bone
develops from the first and second branchial arch, creating the external and middle ear. The inner ear forms from the auditory vesicle, which means that malformations of either do not have to occur simultaneously. Furthermore, malformations of the internal auditory canal (IAC) do not necessarily always have to be associated with malformations of the inner ear, although they might all be associated. According to the literature, 39% of children with hearing loss have some type of malformation in the ear that is visible on CT, and between 21% and 33%, in the inner ear (Fig. 2):

1. **Outer ear malformations.** These have an approximate incidence of between 0.7 and 2.3 per 10,000 births. They are often unilateral and are associated with various malformations of the middle ear, and many syndromes.

2. **Middle ear malformations.** Isolated malformations of the ossicles and of the wall structure are associated, for the most part, with alterations of the external auditory canal (EAC) such as stenosis or atresia and are very rare in isolation. The incus and the stapes are most affected, the malleus more rarely. There is a clinical picture termed “late onset” which occurs at 25 months, the age at
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which the bone marrow becomes bone. In this case, the marrow is reabsorbed and creates a large medullary cavity in the incus and the malleus. This usually occurs in Treacher-Collins syndrome and trisomy 13.\textsuperscript{39} Ossicular chain disorders can be associated with disorders of the facial nerve especially in relation to its position, most often crossing the oval window and fixing to the stapes or together with oval window agenesis, although isolated atresia can occur.

3. Inner ear malformations. The vestibular aqueduct is one of the last structures to develop in the inner ear. It is the most common cause of inner ear malformations in children, at 42.9\% of cases.\textsuperscript{39} It is considered dilated when it measures more than 1.5 mm, which occurs when it is greater than the diameter of a normal posterior semicircular canal (Fig. 3). Since high-resolution CT has been in existence, it has been demonstrated that it is very common for these to exist alongside other cochlear disorders, in up to 100\% of cases according to different series.\textsuperscript{40} Anomalies of the cochlear aqueduct are very rare.

- Anomalies of the cochlea. These are traditionally classified as Michel’s aplasia, Bing-Siebenmann aplasia, Mondini aplasia, Schiebe aplasia and Alexander aplasia. Some authors believe that these can be reduced to Schiebe and Mondini aplasias, but there are numerous disorders that do not fit into any of these dysplasias. Schiebe’s dysplasia is the most common anomaly of the inner ear. The lesions are found in the saccule and cochlea, with atrophy of the vascular stria, deformation of the tectorial membrane and poor differentiation of the organ of Corti, the Reissner membrane collapsing. Mondini’s disorder consists of the lack of development of one of the turns, hypoplasia of the modiolus and absence of interscalar septum; it is caused by interrupted embryological development in the seventh week of gestation.

- Anomalies of the bony or membranous labyrinth. Malformation of the semi-circular horizontal canal is the most common disorder in this group. Anomalies of other isolated canals are rare, with no association with the semi-circular horizontal canal. The most severe malformations of the semi-circular canals are usually associated with a dilated vestibule and cause a semi-circular duct cavity with the utricle and the saccule, absorbing one or all the semi-circular canals. These disorders can be unilateral or bilateral and, if they exist, do not always cause hearing loss, and can produce asymmetric hearing loss. We can also encounter dehiscence of the superior semi-circular canal. Isolated lesions of the utricle, the saccule and the vestibule are rare. There are other disorders of the cochlea, in addition to the various classifications, that cannot be classified, such as dwarf or hypoplastic cochlea with a normal number of turns. There can be complete aplasia, a common cavity or hypoplasia associated or otherwise with semi-circular canal disorders.

- Anomalies of the internal auditory canal. An IAC calibre of less than 2 mm is considered pathological, it can be stenotic, atresic or divided by bone septa. IAC disorders can also be associated with aplasia, hypoplasia or facial nerve duplication. Tumour lesions can be found amongst the acquired lesions, as in neurofibromatosis type II. Aplasia of the cochlear nerve is the most common cause of unilateral sensorineural deafness in children, tumour lesions being rare.\textsuperscript{45} Central lesions that are single or associated with vestibulocochlear nerve and facial nerve lesions are rare and can be identified by MR.\textsuperscript{42}

There are two imaging techniques for the study of congenital child deafness: CT and MR, although other techniques should be considered, such as positron emission tomography (PET) that gives us functional images, which in certain cases can be important in making therapeutic decisions. CT is currently essentially used in the diagnosis of middle and outer ear malformations. There are 2 types: (a) Multi-slice CT (MSCT), for single-plane imaging, and (b) Cone Beam CT (CBCT), which can achieve data in 3D and make reconstructions on any plane. In recent years this has become the CT scan of choice, because exposure time is less, it has higher spatial resolution and the child is subjected to lower radiation. Its disadvantage is its greater sensitivity to the patient’s movement. MR is used in diagnosis of the middle ear, the cerebellopontine angle and to diagnose cholesteatoma of the middle ear. There is no consensus on the selection of the type of sequences for the diagnosis of temporal bone lesions.

Laboratory Tests

Laboratory tests should be used to confirm or support the hypotheses that arise from the first aetiological approach to diagnosing hearing loss. In addition to the abovementioned search for infectious agents, there are other useful analytical tests for diagnosing deafness. For example, in suspected cases, thyroid metabolism should be considered in older children associated with Pendred syndrome. We should not forget to check (if they are included in the history) ototoxic drug levels (e.g., aminoglycosides/vancomycin) in cases of neonates treated with them. A study of the urine of older children might be helpful in relation to Alport syndrome. Other tests, such as insulin resistance, associated with Wolfram syndrome, or renal and parathyroid function tests in hypoparathyroidism, sensorineural deafness and renal disease (HDR syndrome), amongst others, should be guided by clinical suspicion.
Other Tests
Related to long QT syndrome, Jervell and Lange-Nielsen Syndrome (JLNS) is a recessive autosomal variant of family long QT syndrome (LQTS), characterised by profound congenital sensorineural hearing loss, a long QT interval on electrocardiograms (ECG) and ventricular tachyarrhythmias. Its prevalence is unknown and varies according to the population studied (1:200,000–1:1,000,000). Symptoms return after 3 years in practically 50% of patients. The typical presentation of JLNS is a deaf child with episodes of syncope at times of stress, exercise or fear. Deafness is congenital, bilateral, profound and sensorineural. The QT interval in JLNS is markedly prolonged (>500 ms) and is associated with tachyarrhythmias that can cause syncope or sudden death. JLNS is caused by compound homoygous or heterozygous mutations in the KCNQ1 gene (locus LQT1; 11p15.5) or in the KCNE1 gene ( locus LQT5; 21q22.1–q22.2), and is an inherited recessive autosomal disease.

A third of the children with hearing loss present disorders on ophthalmological examination, which can also contribute to an aetiological diagnosis of deafness, therefore this test should always be performed.

Discussion

This document proposes a designed protocol to serve as a guide for professionals to establish the cause of confirmed hearing loss in children. A sequential approach is proposed for the aetiological diagnosis of hearing loss according to the most prevalent causes. Fig. 4 shows an inverted pyramid, with different blocks containing the various diagnostic tests. The size of each block represents, on the one hand, the diagnostic yield of the test (understood as the proportion of relevant results) and on the other, the volume of children that would be tested using this method.

The approach starts with ‘’first diagnostic level’’ actions and tests: clinical history and physical examination. The family history is assessed and the risk factors for hearing loss, for which correct anamnesis is essential, a detailed family tree is made, whenever possible, and the patient is examined physically for signs and stigmata that might indicate a syndrome. Without doubt, the inclusion of data on perinatal and postnatal history (paying special attention to the risk factors of deafness) is essential to achieve the best diagnostic yield of the entire sequence of tests in this block. Expected yield is 41% for family history, 65% for risk factors of deafness and 21% for examination for craniofacial anomalies and syndrome stigmata.39

The ‘’second diagnostic level’’ involves different methods of genetic testing. The extreme genetic heterogeneity of deafness has historically posed a challenge in implementing genetic diagnostics into clinical practice. However, the benefits of achieving an aetiological diagnosis are irrefutable, because it gives us prognostic and reproductive information, helps to reduce anxiety in the patient and their family, enables us to rule out or predict potentially serious syndromic manifestations, prevents unnecessary diagnostic tests and is occasionally useful in therapeutic decision-making.3,21,43 The latter point is increasingly relevant, both due to the influence of some genetic disorders on the performance of cochlear implants, and the fact that a genetic diagnosis is the first step towards future targeted pharmacological intervention or possible genic and cellular therapy options,44,45 which have already yielded their first fruit in hereditary blindness.46,47 More than 80 genes are known and more than 1000 different mutations capable of causing non-syndromic sensorineural hearing loss.48 In our environment, GJB2 mutations and GJB6 deletions as a whole constitute the most frequent cause of hereditary hearing loss.49 Alterations in these genes explain between 10% and 50% of deafness of genetic origin, this percentage depends on the population under study and the clinical features of the patients assessed.19,35 Analysis of genes GJB2 and GJB6 is therefore an essential part of the diagnostic process of child deafness.4 The remaining cases are a consequence of mutations in dozens of different genes, each responsible for a small percentage of families.19,31 These cases would be candidates for broader techniques, such as gene panels performed with NGS studies. Necessarily specialists requesting NGS should be familiar with the limitations of this technology, and select the most appropriate methodology.19 The genes included, the sensitivity and the specificity of the panel and its capacity to detect variations in the number of copies, are the variables that should be taken into account when requesting and evaluating the results of a specific panel.17 A knowledge of the genes that have been analysed in the requested panel is crucial to be able to understand the scope of a negative test. The design of a panel can include from dozens of genes associated with non-syndromic sensorineural deafness, to hundreds of genes responsible for different syndromes or even genes whose association with hearing loss in humans is still under study. At this time there is no consensus about the genes that should be included in a panel for diagnosing hereditary deafness, or the syndromes that should form part of such a panel.17 This means that the number of genes of the different panels vary from a few dozen to more than 200. However, there is consensus that if the diagnostic yield of panels is to be maximised, at least the most common syndromes with variable expressivity should be included.6,30,31 It should not be forgotten that approximately 30% of
sensorineural hearing loss are syndromic in nature and, in some syndromes, the non-audiological signs and symptoms can be very subtle, especially during the first years of life. Even mutations in genes associated with syndromes such as the Usher, Wolfram, Stickler or Pendred syndromes may not have syndromic manifestations.\textsuperscript{2,23} Moreover, on occasion genetic diagnosis might be the only indication of potentially fatal syndromes such as Jervel Lange-Nielsen syndrome, which can occasionally provide electrocardiographic recordings that appear normal.\textsuperscript{44} Furthermore, the panel chosen should be subject to continuous review, since 1 or 2 genes are discovered every month, whose mutation can cause perceptive hearing loss. In fact, more than 25\% of the genes currently involved in sensorineural hearing loss have been discovered in the last 5 years by NGS technology.\textsuperscript{26} It should be taken into account that there are different methodologies, both for isolating the genetic regions to be analysed and for their sequencing. While the sensitivity and specificity of Sanger sequencing is excellent and considered the benchmark, NGS can be contrasted for each panel, it being possible to achieve levels >99\%. Therefore, the panel selected must guarantee sensitivity and specificity levels equivalent to those of sequencing.\textsuperscript{27,28,35}

Another variable that is becoming increasingly important is the analysis capacity to detect not only occasional mutations, but also variations in the number of copies of the genes studied. At least 15\% of the mutations capable of causing hearing loss are the consequence of large deletions or amplifications, variants that are not detected by Sanger sequencing and that require specific NGS technique to be identified.\textsuperscript{14,54,56} At present, given the poor diagnostic yield of the complementary tests, their possible disadvantages (pain, sedation, irradiation, use of contrast media, etc.) and the efficiency of genetic studies, the indication for non-genetic tests should be assessed in each individual case and, unless there is strong clinical suspicion, should be postponed until the results of the genetic studies have been obtained.\textsuperscript{6,10,58,59}

In this regard, and if it is not already available, it is recommended that a test for cytomegalovirus (CMV) is performed in combination with genetic testing, because this virus is one of the most frequent causes of deafness, occasionally postnatal and progressive, and because it is not included in the routine testing of pregnant women. It is important to emphasise that CMV infection does not exclude the possibility of genetic alterations presenting simultaneously associated with hearing loss, as has been demonstrated in some studies.\textsuperscript{60,61} As with congenital infection, diagnosis of CMV infection is based on isolating the virus or identifying its genome by PCR in various biological samples.\textsuperscript{6,62,63} The advantages of PCR include the small amount of sample required and the short time required to obtain results (24–48h). Methods of simple amplification in urine have even been developed that take only 1h for the result, which enables immediate diagnosis of the patient and could be very useful in the study of neonates presenting altered hearing screening, performed with automated potentials.\textsuperscript{64} A diagnosis of CMV might be of particular interest in children who do not pass neonatal screening and are referred to the ENT specialist for confirmation under 2–3 weeks of life, given that most studies have concluded that treatment of CMV is effective if given before one month of life and is prolonged over several months, at least between 6 and 12 months.\textsuperscript{65} Recently data have been published that demonstrate that CMV screening in saliva targeting neonates who have failed neonatal hearing screening is cost-effective, enabling a saving of more than 50\% of the cost.\textsuperscript{66–68} Universal screening for CMV in urine (more exact) or saliva (more practical) is under debate.\textsuperscript{69–72} Based on the high prevalence of the infection and the possibility of improving prognosis with appropriate management and treatment.\textsuperscript{73} Universal screening for CMV would pick up neonates who are not likely to be diagnosed because they are infected asymptomatically, have had a normal first hearing test result and might present late-onset deafness.\textsuperscript{74} Unlike congenital CMV infection, acquired infection in the neonate and infant can appear not to be associated with deafness or with long-term neurodevelopmental disorders. Therefore, precise identification of the time of infection is important using PCR in biological samples at 3 years of life or in dry blood from the sample for metabolopathies, although its sensitivity is lower (approximately 35\%) and therefore a positive result will confirm infection, however, a negative result will not completely rule it out.\textsuperscript{75,76} Although drug treatment is debated, simply knowing about the infection will enable appropriate follow-up of these children and the possibility of early diagnosis of deafness. Given that deafness due to CMV presents in symptomatic and asymptomatic children, is fluctuating and frequently postnatal, these children should be followed up for at least 6 years, with more frequent follow-up of those most affected. Vaccination against CMV is under development and this might change the current situation regarding this disease.\textsuperscript{77,78}

The “third diagnostic level” comprises imaging tests. It is increasingly frequent to perform CT scans on children for various reasons, which increases the risk of cancer through their lifetimes. It is calculated that there is a cancer risk of 0.07\% due to exposure of the head to CT in a child aged one year. Although this is apparently a low figure, it is calculated that 500 children die each year in the United States due to radiation by CT performed under the age of 15 years.\textsuperscript{79–81} It is very important to be aware that we should choose imaging techniques in children with care in order to prevent side effects, which although rare, can cause mortality in significant number of cases. It should be borne in mind that many children with hearing loss have other associated diseases as well that also require radiological studies. In these cases, coordination should be improved between the different professionals involved in the care of the child to plan tests jointly, especially if they are to be performed on the same area of the body. Furthermore, the test that is going to provide the most information should be established and the best quality CT to reduce radiation and the number of repetitions performed due diagnostic doubt. It should be insisted that a test must be to guide diagnosis and prognosis and above all treatment, and therefore the appropriate age should be chosen in relation to objectives, causing the least damage to the patient. The older the child, the lower the risks of radiation and therefore, for middle and outer ear malformations we must postpone CT until the age of 3 or 4 years, in the best of cases. The study of sensorineural hearing loss should always start with MR. Neither should we forget that small children require sedation to undergo these tests, with the risks and inconvenience that this implies.\textsuperscript{82}
The "fourth diagnostic level" comprises laboratory and other complementary tests. These include: laboratory analysis, aimed at confirming clinical suspicion and associated syndromes, and ECG in patients with arrhythmia and syncope.

In addition to the above, given its relevance, it is important to highlight that an appropriate and full ophthalmological examination is essential in all cases, given that a third of children with hearing loss present disorders during this test which can also give an indication of the aetiology of their deafness.

CODEPEH Recommendations

The recommended sequence for the aetiological diagnosis of deafness, according to the different levels of diagnostic yield, from low to high, is as follows (Fig. 4):

1. **First diagnostic level.** Anamnesis and physical examination. Create a detailed family tree from the family history. Collect data on risk factors for deafness. With the full physical examination, take into account information of stigmata associated with syndromic hearing loss.

2. **Second diagnostic level.** The genetic aetiology should be sought, according to the algorithm in Fig. 1. Refer the patient for genetic counselling. In order to minimise the costs of the process, the first step recommended is to test for the presence of GJB2 mutations and GJB6 deletions. If it is not possible to identify the cause of deafness after analysis of these genes, the next step should be gene panel sequencing. Offer the patient and their family exome sequencing, in order to identify new genes involved in hereditary hearing loss, in cases where a cause for deafness has not been established after an appropriate diagnostic process. Do not forget that a negative result only indicates that a mutation has not been found in the genes analysed, but does not exclude the possibility that the cause of deafness is genetic. The patient’s history should be searched for previous positive CMV PCR assays in the first 3 weeks of life, which would establish the presence of congenital infection. The blood sample taken for genetic study can be used for the CMV PCR assay, if this has not been possible to establish beforehand, bearing in mind that from 2 to 3 weeks of life a positive result for the virus is of value for the diagnosis of unclear congenital infection. In positive cases, the study of congenital infection with CMV should be completed by PCR in biological samples from the first 3 weeks of life which have been stored or in the sample of dry blood from the new-born screening test, if available. In cases that have been confirmed in this manner the usefulness of starting valganciclovir treatment should be assessed. If it is not possible to confirm congenital infection, diagnosis will be presumed and based on additional compatible clinical signs (ocular, brain, haematological problems) in the opinion of the practitioner, who will decide the approach. Children that are congenitally infected by CMV must be monitored for at least 6 years, with more frequent follow-up of the most affected, given that congenital CMV infection, which presents in both symptomatic and asymptomatic children, fluctuates and is often postnatal.

3. **Third diagnostic level.** Both CT and MR are appropriate, and in different situations, complementary methods for the aetiological diagnosis of deafness. Consider the technique that will involve minimal radiation for the patient when choosing the type of test for the diagnostic process. Take the patient’s age into account and the best time to undertake the tests. In disease involving malformations of the outer and inner ear, CT is the technique of choice. It is advisable to wait until the age of three provided it is not required for another reason. It is better to use Cone Beam CT because it emits minimum radiation and is very efficient for diagnosis. MR is the technique of choice in malformations of the inner ear, IAC and brain. Bearing in mind that lesions to the inner ear are the most common cause of sensorineural hearing loss in children, MR should be the first imaging test.

4. **Fourth diagnostic level.** To evaluate thyroid hormones, urine test and other tests geared at detecting specific syndromes, according to clinical suspicion. Consider ECG in children with syncope.

5. **Ophthalmological examination.** Ophthalmological examination is always necessary, it can also indicate specific infections or syndromes associated with deafness.

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Conflict of Interest

The authors have no conflicts of interests to declare.

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