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Genetics of Hearing Loss – Syndromic

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Synopsis

Hearing loss (HL) is one of the most common birth defects in developed countries and is a diverse pathology with different classifications. One of these is based on the association with other clinical features, defined as syndromic hearing loss (SHL). Determining the etiology of the HL in these patients is extremely beneficial as it enables a personalized approach to caring for the individual. Early screening can further aid in optimal rehabilitation for a child's development and growth. Pathogenic variants in forty-five genes, encoding proteins functioning as ion channels, transcription factors, molecular motors and more, are known to lead to eleven forms of SHL. The development of high-throughput sequencing technology is facilitating rapid and low-cost diagnostics for patients with SHL.

Keywords

Deafness; Hearing loss; Genetics; Genome; Sequencing

INTRODUCTION

Hearing loss (HL) is the most prevalent sensory impairment in both childhood and adulthood[1, 2]. According to the last update of the World Health Organization (WHO), approximately 360 million people worldwide, equaling 5% of the world's population, suffer from a disabling HL (Table 1). The majority of these people live in low and middle-income countries, where treatments for HL are more difficult to obtain and consanguinity increases the risk of recessive disease. HL is an etiologically heterogeneous pathology caused by different genetic and environmental factors, with half of the cases estimated to be genetic[3]. HL can also be a result of infections, injuries and exposure to excessive noise.

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HEARING LOSS

Our ability to hear is orchestrated by the auditory system. The vestibular system is responsible for balance, three-dimensional orientation and gravity perception. The ear is a three-chambered organ divided into the external, the middle and the inner ear, which are all essential for the intact activity of the auditory and the vestibular systems. The external and middle ear are responsible for collecting and conducting the sound wave's energy to the inner ear[4,5]. The sensorineural end organ of hearing is the snail-shaped organ of Corti that resides in the inner ear. It is composed of a single row of inner hair cell (IHC), three rows of outer hair cells (OHC) and supporting cells. The IHC act as sensory transducers, capturing stimulus energy, interpreting it as electrical responses and sending the impulses to the brain through the auditory nerve. The OHC are responsible for enhancing the signal[6].

According to the American Speech-Language-Hearing Association (ASHA) (Table 1), normal hearing occurs in the range of −10 to 15 dB, with a slight HL if the range of loss is within 16 to 25 dB. Mild HL occurs when the hearing loss ranges between 26 to 40 dB, moderate HL is when the hearing loss ranges between 41 to 55 dB and moderate to severe HL ranges between 56 to 70 dB. Individuals with HL in these ranges are considered to be 'hard of hearing' and can benefit from hearing aids and assistive listening devices. With severe or profound HL, one is considered to be 'deaf,' when hearing loss ranges between 71 to 90 dB in the former case, or profound, when the hearing loss range is above 91 dB. Individuals with this kind of HL may benefit only from cochlear implants[7].

HL is the most common neurosensory disorder in humans. It can be a congenital pathology, caused by genetic factors or by complications during pregnancy and childbirth. It can also be acquired later in life, at any age. Acquired HL can be caused by infectious diseases, physical injuries, the use of ototoxic drugs and genetic pathogenic variants or mutations. Congenital HL is the most commonly occurring condition for which newborns are screened for, with about 1 out of 1,000 infants born affected. Nearly one in five individuals aged 12 and older suffer from unilateral or bilateral hearing loss in the United States alone[8]. Age related hearing loss (ARHL) is the most prevalent sensory deficit in the elderly[9], with nearly 25 percent of those aged 65 to 74 and 50 percent of those who are 75 and older suffering from a disabling HL in the United States alone, according to the National Institute on Deafness and Other Communication Disorders (NIDCD; Table 1). Half of the HL cases are estimated to be genetically related[3] and account for about 50 to 60 percent of childhood HL cases in developed countries[2]. Over the years more than 100 deafness-related loci and their associated genes have been identified and studied, revealing the genetic basis of different deafness-related pathologies[10].

The diversity of ear disease pathologies is classified according to the etiology of the case, which can be genetically related or due to environmental causes. If the pathology is genetically related, it is further classified according to the pattern of inheritance (dominant, recessive, X-linked or Y-linked). HL is also classified according to the onset of the pathology, the type, the severity, uni- or bilateral and the association with other disorders as syndromic hearing loss (SHL) versus non-syndromic hearing loss (NSHL)[11].

For NSHL, HL loci are classified and named according to their mode of inheritance, with a prefix of DFN (for DeaFNess) (Hereditary Hearing Loss Homepage; Table 1). DFNA refers to autosomal dominant inheritance, DFNB refers to autosomal recessive inheritance and DFNX refers to an X- linked mode of inheritance. Furthermore, Y-chromosome linked genes and maternal inheritance linked to mitochondria have also been identified[12]. Each locus name also contains a number that represents the order in which these loci were identified in association with deafness. In many cases, the genes for the DFN loci have subsequently been identified[13] (Hereditary Hearing Loss Homepage, Deafness Variation Database; Table 1; Chapter this book)

As HL is one of the most common birth defects in developed countries, newborn screening for hearing defects has an important role in treatment and rehabilitation strategies, such as cochlear implantation[14]. The early diagnosis of the etiology of a child's HL can allow the monitoring of possible complications and can indicate which therapy is the most suitable and effective one. It also allows for more accurate genetic counseling for parents who want to have more children[10].

SYNDROMIC HEARING LOSS

SHL is a form of HL accompanied by additional clinical features. Approximately 30% of the genetic cases of HL are considered to be syndromic[11]. SHL consists of HL that presents with anomalies of the eye, kidney, the musculoskeletal and the nervous systems, as well as pigmentary disorders and others[15] (Figure 1). Among the well-known syndromes are Usher, Waardenburg and Pendred. Of these syndromes, Pendred and Usher syndromes are the most common[10]. Several genes associated with SHL (Figure 2) are also involved with NSHL, as in the case of *SLC26A4* mutations leading to Pendred syndrome and DFNB4[10]. The genes associated with SHL are represented in Table 1 and Figure 1.

FORMS OF SYNDROMIC HEARING LOSS

Usher Syndrome

The eye and the ear are the sensory organs responsible for vision, balance and hearing in mammals. These organs are essential for both communication and environmental perception[16, 17]. Diseases affecting the inner ear and retina of the eye can cause major impairments for human communication systems. Syndromes that include symptoms of both blindness and hearing impairment are widely known. In humans there are approximately 40 syndromes that include both impairments, and about half of the affected cases are caused by mutations attributed to Usher syndrome[18]. Usher syndrome is an autosomal recessive genetic disease with clinically and genetically heterogeneous characteristics. In humans it is defined by congenital, bilateral deafness and a later onset of vision impairment, caused by retinitis pigmentosa[16]. Epidemiological studies have estimated that the prevalence of Usher syndrome ranges from 1/6,000 to 1/10,000[19, 20]. Usher syndrome is subclassified into three clinical types, USH1, USH2, and USH3, based on the severity of the sensorineural hearing loss (SNHL), the presence or absence of vestibular dysfunction and the age at onset of retinitis pigmentosa. USH1 patients suffer from severe to profound congenital bilateral HL accompanied by congenital vestibular dysfunction. In terms of retinitis pigmentosa

symptoms, night blindness may be detected during childhood, followed by a narrowing of the visual field, which progresses to severe blindness[16, 21]. USH2 patients suffer from moderate to severe congenital HL with no vestibular abnormalities. Retinitis pigmentosa is usually diagnosed between the ages of 10 to 40[22]. In USH3 patients, hearing impairment begins before the 3rd decade of life and is characterized by variable progression. In most cases the patient eventually becomes profoundly deaf. Vestibular defects are variable and retinitis pigmentosa usually begins from the age of 20[22]. Early symptoms of retinitis pigmentosa are night blindness and loss of peripheral vision. This form of Usher syndrome is the least common in the general population[23], but is more prevalent in the Finnish and Ashkenazi Jewish populations[24, 25].

To date, 16 independent loci on different chromosomes are known to be associated with Usher syndrome. These loci are further divided into *USH1A-G*, *USH2A-C* and *USH3A*. Moreover, 13 genes have been identified. The *USH1* genes are *MYO7A* for the *USH1B* locus, encoding the motor protein myosin VIIA[26]. *USH1C* encodes harmonin[27, 28] and *USH1G* encodes SANS[29], both of which are scaffold proteins. *CDH23* mutations are responsible for USH1D, which encodes cadherin 23[30], *PCDH15* mutations lead to USH1F and encodes protocadherin 15[31], both of which are cell adhesion molecules. *CIB2* mutations are the cause of USH1J, which encodes calcium and integrin binding protein 2[32]. The *USH2* genes are *USH2A*, encoding usherin[33] and *ADGRV1* for USH2C, encoding adhesion G protein-coupled receptor VI, also referred to as *GPCR98* or *VLGR1*[34, 35]. Both genes are transmembrane proteins that are involved in signaling. Another gene associated with USH2 is *WHRN*, encoding whirlin for USH2D[36]. The *USH3* genes are *CLRN1* for USH3A, encoding Clarin 1, and *HARS* (Histidyl tRNA synthetase)[24, 37–39]. Moreover, another two USH genes have recently been identified. *PDZD7* encodes the protein PDZ domain containing 7 and *CEP250* encodes centrosome associated protein 250. Usher syndrome has recently been proposed to be an oligogenic disease, due to digenic inheritance of *PDZD7* and *USH2A* or *ADGRV1* in patients with Usher syndrome. Two genes have been proposed to lead to variable phenotypes of Usher syndrome, depending on the dosage. *CEP250* is associated with early onset HL and severe retinitis pigmentosa in conjunction with two mutant alleles of *C2orf71*. The patients have mild HL and retinal degeneration with one mutant allele of *C2or71*[40, 41]. C2orf71 mutations are associated with retinitis pigmentosa and proposed to encode a ciliary protein[42]. Importantly, many of the genes listed above have been reported to cause NSHL (Figure 1). For example, different mutations in *MYO7A* are known to cause recessive deafness DFNB2 and dominant deafness DFNA11[43, 44].

Waardenburg Syndrome

Waardenburg syndrome was considered to be an autosomal dominant inherited disease of the neural crest cells, but this syndrome is more clinically and genetically heterogeneous than originally known[45]. Waardenburg syndrome is characterized mostly by SNHL and pigmentation abnormalities that can occur in the eyes, hair, skin and the cochlear stria vascularis. Other features can be found in a subset of patients. These features are used for clinical classification of the syndrome. Waardenburg syndrome is estimated to have a prevalence of 1/42,000 and is responsible for 1–3% of all congenital HL cases[45]. During embryonic development, the pluripotent neural crest cells migrate from the neural tube and

give rise to different cell types, among them, melanocytes of the skin and inner ear, glia, neurons of the peripheral and enteric nervous systems and some of the skeletal tissue. The symptoms associated with Waardenburg syndrome results from an abnormal proliferation, survival, migration or differentiation of neural crest-derived melanocytes[45]. Waardenburg syndrome is subdivided into four subtypes, WS1, WS2, WS3 and WS4, on the basis of the presence or absence of additional symptoms[46]. WS1 is further characterized by dystopia canthourm, an appearance of wide-set eyes due to a prominent broad nasal root, while WS2 has no further significant features. WS1 and WS2 are the most frequent among the four subtypes. WS3 is further characterized by dystopia canthorum and musculoskeletal abnormalities of the upper limbs. WS4 is associated with Hirschsprung disease, characterized by a blockage of the large intestine due to improper muscular bowel movement and neurological defects. Neurological features were also observed in a subset of WS2 patients[47]. Among the symptoms of this syndrome, the SNHL is the most frequent one with 60% in WS1 to 90% in WS2[48]. Six genes are associated with this syndrome: *PAX3* (Paired box 3)[49], *MITF* (Microphthalmia-associated transcription factor)[50], *EDNBR* (Endothelin receptor type B)[51], *EDN3* (Endothelin 3)[52], *SOX10* (SRY box10)[53], and *SNAI2* (Snail homolog 2)[54]. These genes are known to be involved in the regulation of melanocyte differentiation[45]. A database for the Waardenburg syndrome-associated genes can be found at the Leiden Open Variation Database (Table 1).

Pendred syndrome

Pendred syndrome is one of the most common autosomal recessive syndromic causes of HL. The audiological phenotype is quite broad, ranging from mild to profound and can be congenital or with a later onset and be progressive[55]. A common feature among patients is an enlarged vestibular aqueduct (EVA), a common radiological malformation of the inner ear. In addition to SNHL, patients who suffer from this syndrome also show features of congenital and severe to profound temporal bone abnormalities, in addition to goiter partial iodine organification defects resulting in a positive perchlorate discharge test from the goiter, usually in late childhood to early adulthood. Pendred syndrome also features thyroid dysfunction, ranging from euthyroid to hypothyroidism[56, 57], and vestibular dysfunction, demonstrated in approximately 65% of affected individuals. The vestibular dysfunction can range from mild unilateral canal paresis to gross bilateral absence of function[58].

The estimated prevalence of Pendred syndrome is 7.5 per 100,000 newborns and it accounts for approximately 1 to 8% of the cases of congenital deafness[59]. Approximately half of the Pendred syndrome cases are caused by a mutation in one of three genes. *SLC26A4*, encoding the protein pendrin, is an iodide-chloride transporter. Mutations in this gene are responsible for both Pendred syndrome and DFNB4, a form of NSHL. Pendrin is expressed in the kidneys, the inner ear and thyroid[59]. Approximately 50% of Pendred syndrome affected individuals have a mutation in this gene (Genetics Home Reference, Table 1). Less than 2% of the rest of the affected individuals have mutations in *FOXI1* encoding Forkhead box protein I1 or *KCNJ10* encoding the ATP-sensitive inward rectifier potassium channel 10[58]. More than 280 *SLC26A4*-Pendred syndrome and DFNB4-causing mutations have been identified, but in different ethnic groups, unique pathogenic alleles are found more frequently than others, reflecting a few prevalent founder mutations[58].

Additional syndromes

In addition to the above-mentioned syndromes, there are over 700 genetic syndromes that have been described with features of hearing impairment[15]. Alport syndrome is characterized by renal defects, SNHL and ocular abnormalities[60] with a prevalence of 1 in 50,000 (Genetics Home Reference).Three genes are associated with this syndrome: *COL4A3* encoding collagen, type IV, alpha 3, and *COL4A4* encoding collagen, type IV, alpha 4 for the autosomal inherited types[61, 62] and *COL4A5* encoding collagen, type IV, alpha 5 for the X-linked type[63].

Branchio-oto-renal (BOR) syndrome is an autosomal dominant disease, characterized by defects in the development of the tissues in the neck and malformations of the ear and kidney[64]. It is estimated that the prevalence of this syndrome is 1 in 40,000 (Genetics Home Reference). Approximately 40% of the individuals affected test positive for mutations in the *EYA1* gene encoding the eyes absent homolog 1. An additional 5% and 4% of affected individuals have a mutation in *SIX5* encoding the homeobox protein SIX5 and *SIX1* encoding the homeobox protein SIX1, respectively[64].

CHARGE syndrome is an autosomal dominant syndrome that features coloboma, heart defects, choanal atresia, retardation in growth and development, genital abnormalities, hearing loss and vestibular dysfunction. It is estimated that the prevalence of CHARGE syndrome is 1 in 8,500 to 10,000 individuals (Genetics Home Reference). This syndrome is mostly caused by mutations in the *CHD7* gene encoding Chromodomain-helicase-DNA-binding protein 7, an ATP- dependent chromatin remodeling protein[65].

Jervell and Lange-Nielsen syndrome is an autosomal recessive disease with features of arrhythmia, SNHL and a significantly higher risk of fainting and sudden death as a result of prolongation of the QTc interval[66]. This syndrome is estimated to affect 1.6 to 6 per 1,000,000 people worldwide, with a higher frequency in Denmark (Genetics Home Reference; Table 1). The genes associated with this syndrome are *KCNQ1* encoding the potassium channel, voltage gated KQT-like subfamily Q, member 1 and *KCNE1* encoding potassium channel, voltage gated subfamily E regulatory beta subunit 1[66–68]. Approximately 90% of the cases are caused by a mutation in the *KCNQ1* gene, with the rest of the cases caused by mutations in *KCNE1*[69, 70].

Norrie disease is characterized by a spectrum of fibrous vascular changes of the retina at birth that progresses to visual impairment with age. About 30 to 50 percent of males with Norrie disease have developmental delays or other forms of intellectual disability, behavioral abnormalities or psychotic-like features. Moreover, the majority of the males also develop HL. This syndrome is X-linked, recessively inherited and caused by mutations in the *NDP* gene encoding the norrin protein. Mutations in this gene are responsible for about 95% of the affected individuals[71]. The prevalence of this syndrome is unknown and it is not associated with any racial or ethnic group (Genetics Home Reference, Table 1).

Stickler syndrome can be both dominant and recessive and is characterized by ocular, skeletal, orofacial and auditory abnormalities[72, 73]. The prevalence of this syndrome is about 1 in 7,500 to 1 in 9,000 newborns (Genetics Home Reference; Table 1). Stickler syndrome is

subdivided into five subtypes based on its underlying genetic collagen defect. For the autosomal dominant form of Stickler syndrome, three genes have been identified. Type I Stickler syndrome (STL1) is associated with mutations in *COL2A1* encoding collagen, type II, alpha-1[74]. Moreover, mutations in *COL11A1* encoding collagen, type XI, alpha-1 are associated with type II (STL2)[75] and mutations in *COL11A2* encoding collagen, type XI, alpha-2 are associated with type III (STL3)[76]. The autosomal recessive forms of Stickler syndrome are STL4 and STL5, and their identified related genes are *COL9A1* encoding collagen, type IX, alpha-1 and *COL9A2* encoding collagen, type IX, alpha-2, respectively[77, 78]. There is a degree of variability in HL frequency and severity in the different types of this syndrome, even within the same family[79].

Treacher-Collins syndrome is usually an autosomal dominant syndrome that affects the development of the bones and other tissues of the face. These abnormalities contribute to speech and language difficulties, visual impairment, conductive HL and breathing difficulties. The symptoms of this syndrome can range from undetectable to severe. Half of the affected individuals suffer from HL caused by defects of the three bones of the middle ear or defects in the development of the ear canal. One in 50,000 people will suffer from this syndrome (Genetics Home Reference; Table 1), caused by mutations in three genes: *TCOF1* encoding the treacle protein, *POLR1C* encoding polymerase I polypeptide C and POLR1D encoding polymerase I polypeptide D. The majority of patients have a mutation in *TCOF1*, with 1% of the cases caused by a recessive form of this syndrome, with mutations in *POLR1C*[80].

Perrault syndrome is an autosomal recessive disease characterized by SNHL in both sexes and ovarian dysfunction in females. The HL symptoms are bilateral and ranges from moderate with early childhood onset to profound at birth. Moreover, the early childhood form can be progressive. The ovarian dysfunction symptoms can also vary and affected females also show, in some cases, neurological features, such as developmental delay and cerebellar ataxia. Less than 100 affected individuals have been documented (Genetics Home Reference; Table 1), most probably due to difficulties in diagnosis. Four genes have been associated with this syndrome: *HARS2* encoding Histidyl-tRNA Synthetase 2, HSD17B4 encoding hydroxysteroid (17-beta) dehydrogenase 4, LARS2 encoding leucyl-tRNA synthetase2 and CLPP encoding the caseinolytic mitochondrial matrix peptidase proteolytic subunit. This syndrome is subdivided into 4 types: type I, II, III and IV, also called PRLTS1, 2, 3, and 4, respectively. The classification of the subtypes is determined according to the neurological involvement and its state, progressive or non-progressive. This classification is now being reconsidered as mutations in *CLPP* were found to include both types of cases, with or without neurological symptoms[81]. The clinical features and the molecular genetics information of these syndromes are comprehensively described in OMIM, the Online Mendelian Inheritance in Man database (Table 1).

GENETICS OF HEARING LOSS

Single-gene disorders may be inherited in an autosomal recessive or dominant mode, be carried on the X-chromosome or inherited through the mitochondria. Each form of inheritance may have implications for the number of children to manifest the disease in each

case, or whether one or both sexes will be affected. As a result, an accurate and thorough family history is essential upon examining the patient.

Diseases inherited in a recessive manner are often the most severe in nature and are expressed at birth (congenital) or soon thereafter. As recessive inheritance 'skips' a generation, and depends on two carriers of the pathogenic variant, there is often no family history in patients with a recessively inherited disease (Figure 3A). In other cases, multiply affected patients in every other generation are very clear signs of recessive diseases (Figure 3B). Consanguinity, where parents are related, may increase the incidence of recessive disease and is more prevalent in regions of the world such as the Middle East, India and Pakistan (Figure 3A, B). Both females and males are affected in equal proportions. Many forms of SHL are inherited in a recessive manner, including Jervell and Lange syndrome, Usher syndrome, Pendred syndrome, Perrault syndrome and some forms of Alport and Waardenburg syndrome (Table 2).

Dominant inheritance tends to involve onset of the disease phenotype later in life and may be less severe than recessively inherited diseases (Figure 3C). Only one parent need to carry the pathogenic variant and is affected by the disease as well. Complications in diagnosis may arise due to reduced penetrance, whereby a patient harbors the genotype but not the phenotype of the disease.

It is important to note that several syndromes may be inherited in either a recessive or dominant fashion, depending on the gene and variant involved. Furthermore, families may harbor mutations in more than one gene, leading to the presence of more than one disease in an extended family (Figure 3D).

A more complex scenario arises due to the oligogenic nature of some diseases. It has become clearer in recent years that the phenotype of patients, even with what appears to be a single-gene disorder, may be due to multiple variants. An example is described above for Usher syndrome[40, 41].

DIAGNOSIS OF HEARING LOSS

Screening and identifying the etiology of HL is extremely important to provide the best opportunities for care and rehabilitation in children. The introduction of newborn screening for HL in developed countries has led to earlier diagnosis and improvement in ascertainment and potential outcomes[82], as treatment strategies are examined and applied sooner. Determining the etiology of HL is even more crucial in cases of SHL, as the associated clinical features are usually more severe[83]. Early diagnosis can help predict the progression of the HL in the patient and the prescribed course of action in treating the patient, as well as provide warnings for future potentially life threatening abnormalities.

A continuing challenge in medical genetics has been to determine the etiology of each disease. For example, the presence of *SLC26A4* mutations in a child may help predict whether the child will develop goiter and hypothyroidism after puberty, or be more susceptible to acute HL following head trauma[83]. Patients with Jervell and Lange-Nielsen syndrome will be more sensitive to syncope and are at risk for sudden death[83]. As a result,

being aware that a child has a *KCNQ1* mutation can be extremely informative for his or her health. Today, mutations in at least 45 genes are known to be associated with SHL, providing an opportunity for patients with these mutations to benefit from this knowledge. Most of these discoveries have only been made in the last two decades, and some even more recently. Despite this progress, the underlying genetic cause of hundreds of inherited syndromes is still unknown and the continuing challenge is to uncover the etiology for the affected children and adults.

Identifying disease genes through linkage mapping with genetic markers

The large size of the genome and the high number of genes it contains made the identification of disease-associated genes a tedious task from a historical perspective. Before the Human Genome Project (HGP) was completed in 2001[84], researchers used various techniques for identifying and mapping genes associated with Mendelian and single-gene inherited disorders. Linkage mapping, the most common technique used, was aimed at finding the approximate location of the disease gene relative to a DNA segment with a known chromosomal location, a genetic marker[85]. The process included scanning the genomes of members of an affected family and using highly polymorphic DNA markers to identify regions linked with the disease. The affected family members shared specific variants of the DNA markers more frequently than in the general population, suggesting linkage with the presence of a nearby disease gene. To focus on the disease gene, multiple markers in the linked region were further genotyped to define the critical region. DNA markers were generally in the form of microsatellites, repetitive DNA elements present every 1000 bp in the human genome. Their polymorphic nature and presence of multiple alleles in the population rendered these markers to be extremely useful. The most commonly used ones were CA repeats, which could be purchased commercially and could be used for automated genotyping as they were labeled with fluorescent markers. The next generation of DNA markers involved single nucleotide polymorphisms (SNPs). Although they are less polymorphic, having only two alleles per SNP, they are more frequent in the genome, at every 100–300 bp, and facilitate linkage analysis. As most of the human genes are annotated, examination of the region using genome browsers, such as the UCSC Genome Browser (Table 1), can provide a list of the genes in the region. The challenge is then to identify the pathogenic variant that leads to the disease under study. Oftentimes there was a gap of years from identification of the chromosomal location of the putative gene, to identification and validation of the pathogenic variant. For example, a number of families with USH1 from Pakistan were studied by linkage analysis with microsatellite markers[86]. Years later, one of the families in this group was found to harbor a mutation in *CIB2*, defining a new form of Usher syndrome, USH1J (pedigree in Figure 3B). The majority of genes for SHL were identified in this way.

Validation of the pathogenic variant is subsequently performed using capillary Sanger sequencing, which allows for sequencing of regions of approximately 800–1000 bp. A comparison of the patient's sequence to unaffected family members can help define the critical variant. However, additional criteria are required for the variant to be defined as the disease causing change in the DNA. Functional analysis, by replicating the variant in cell culture, may help define the pathogenicity further. For example, the *SLC26A4* variant c.

1458_1459insT, when replicated in COS7 cells, was mislocalized, providing further evidence that this is a pathogenic variant[87]. Finally, reduction of a particular gene in an animal model can provide the most compelling circumstantial evidence of pathogenicity, as well as demonstrate the mechanisms involved. *CIB2*, a candidate for Usher syndrome, was shown to be essential for mechanosensory hair cell function in zebrafish[32]. Gene-targeted mutagenesis of *Slc26a4* in the mouse revealed that Pendrin, the protein encoded by this gene, is important for the development of the inner ear and provided an understanding of the cause for deafness in Pendred syndrome patients[88]. An additional *Slc26a4* mutation in mice, *Slc26a4* $^{loop/loop}$, had symptoms of deafness but without the enlarged thyroid gland symptoms that characterizes Pendred syndrome[89]. However, histological analysis of the thyroid tissue showed morphological defects and the inner ear showed molecular and morphological defects consistent with the symptoms seen in patients with disrupted thyroid hormone activity. This study led to the proposal that thyroid hormone deprivation, caused by this *Slc26a4* mutation, contributes to the deafness in this mouse model.

Identifying disease genes through high-throughput sequencing

The development of high-throughput sequencing technology has revolutionized both discovery and diagnosis of pathogenic variants for disease. Massive parallel sequencing (MPS), also known as Next Generation Sequencing (NGS), enables a more rapid and low-cost method of DNA sequencing. Compared to the traditional capillary or Sanger sequencing, these methods perform large-scale sequencing, generating a billion bases within a single run[90]. The MPS output is aligned to the human reference genome, a consensus representation of the genome (Genome Reference Consortium; Table 1), enabling the identification of differences between the reads of the current genome being sequenced and the reference genome. Bioinformatics tools, such as the Genome Analysis Toolkit (GATK) software package (Table 1), are next used for detecting the variants, examining whether the variant is unique, the frequency of this variant, and the potential damage of this variant to the protein structure and function. Moreover, the sequence conservation is also examined as well as the mode of inheritance, which should match the one of the disease analyzed. The potential pathogenic variants are then screened for segregation in the affected family. This analysis is less complicated if the variant detected is a known deafness mutation, while variants in genes not previously associated with deafness are more challenging to prove[91].

The development of MPS changed the field of gene sequencing, enabling multiple genomes to be sequenced simultaneously. However, the complexity of genomic analysis for the large number of variants that arise, in particular from non-coding regions of the genome, remains challenging. To circumvent this problem, researchers studying highly heterogeneous diseases such as Usher syndrome[92, 93], inherited eye diseases[94], or deafness[95, 96], have screened only for genes already known to be associated with the disease under study. In cases where pathogenic variants are not found using a select number of genes, Whole Exome Sequencing (WES) becomes the next option. As the costs are decreasing, WES is sometimes the first approach[97]. In this method, only coding exons of genes are sequenced, alleviating the need to analyze non-coding regions, with a substantial savings of cost. Whole Genome Sequencing (WGS) has been used for identification of human disease genes in severe cases of neonatal disease[98]. Though considerably more costly, WGS is considered to

be the most comprehensive method for detecting all variants, in particular copy number variants (CNV) that may be involved in disease[98]. Furthermore, linkage analysis, described above, may be used in conjunction with WGS to facilitate localization of the pathogenic variant[98]. Overall, currently, NGS technology has a molecular diagnostic success rate of 25%[99] and is predicted to solve the genetic basis of 60% of Mendelian inherited disorders in the near future[100].

The importance of genetic research in understanding the processes involved in hearing and deafness

To date there are a number of different treatments available for hearing impairment. These include amplification of sound using hearing aids and cochlear or auditory brainstem implants that stimulate the cochlear nerve or the nuclei, respectively[11]. Although the physical structure and activity of the ear are well understood, the specific part of the ear that each deafness-associated gene functions in is not fully characterized. Defining the genes and pathways responsible for normal hearing are paving the way towards developing new treatments for hearing impairments. Among the different approaches for newly developed hearing impairment treatments are the regeneration of inner ear sensory cells[101, 102] and the use of viral vectors for gene therapy[103]. These potential treatments will be helpful for both genetically and environmentally-related HL[11].

PROSPECTS OF FUTURE TREATMENT MODALITIES FOR HL

Although hearing aids and cochlear implants are far from being ideal, HL treatments today rely mostly on these approaches. With the aim of providing more optimal treatments for HL patients, the research for alternative treatments has progressed toward different fields, including the use of gene therapy. Understanding the genetic basis of the HL and advances in DNA delivery methods are both essential for achieving the goal of gene therapy development for HL and for others disorders as well[104]. One approach for HL therapy is the use of antisense oligonucleotides. These were successfully used in a mouse model with a loss of function mutation in *USH1C*, which correlates with the human Acadian *USH1C* mutation[105]. This specific mutation leads to defects in splicing of the *USH1C* messenger RNA (mRNA), encoding the harmonin protein. Antisense oligonucleotides can modulate posttranscriptional regulation by gene silencing or alteration of RNA metabolism[104]. In this case the oligonucleotides were used to restore the correct splicing of the gene, enabling expression of the protein. The most effective results were achieved when 3 to 5 day old mutant mice were injected intraperitoneally with the antisense oligonucleotides. Although high-frequency hearing was not developed in these mice at this stage, low and mid-frequency hearing levels were the same as those measured in non-mutant mice. These results were maintained for six months[105]. Implementing this treatment for humans is more complex. Human newborns can hear, which might require gene therapy intervention during gestation. Moreover, the delayed onset of hearing development in mice makes comparisons to humans difficult. Nevertheless, these initial results are promising, providing hope for the future[104,106].

Another possible approach to rescue HL is the use of hair cell regenerative treatments, as most HL cases are caused by irreversible damage to hair cells[106]. As mammalian cochlear

hair cells do not regenerate naturally, different strategies are being employed to regenerate or transplant hair cells. The basis of regenerative research relies on the observation that hair cell regeneration is possible in birds, fish and amphibians[101]. Regenerative research is aimed at discovering the exact formula of reagents that will trigger the regeneration of mammalian cochlear hair cells from precursor cells. The field of transplantation focuses on coaxing either inner-ear stem cells or even pluripotent stem cells into hair cell lineage. These cells may subsequently be transplanted into the inner ear. Although very promising, both strategies are in very early stages for HL treatment[106, 107]. Novartis and Genvec are currently implementing the first clinical trial using the *Atoh1* gene, which can induce the differentiation of sensory cells in the inner ear (Table 1).

Efforts are also being made in the pharmacological field with the aim of discovering novel drugs for HL. This field is not limited to orally delivered pharmacological compounds. The use of non-invasive intra-tympanic or invasive intra-cochlear routes are options being examined as well[108]. Animal models, including zebrafish and mice, are widely used as preclinical models for drug use, both *in vivo* and *in vitro* with cochlear cultures[109, 110]. While there is potential in HL drug treatments, to date, there are no Food and Drug Administration (FDA) approved drugs on the market[106].

Summary

Determining the etiology of deafness in a patient with SHL is a key goal in order to provide optimal rehabilitation options. In the future, understanding the mechanisms of HL due to each genetic mutation will pave the way for therapeutic delivery. Alleviating the isolation caused by deafness will greatly improve the quality of life of these patients. The recent development and implementation of new high-throughput sequencing technology will facilitate a significant improvement in identification of novel syndromic and non-syndromic HL-associated pathogenic variants and genes.

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Abbreviations

ARHL	age related hearing loss
ASHA	American Speech-Language-Hearing Association
ATP	adenosine triphosphate
BOR	Branchio-oto-renal
bp	base pairs
CHARGE	Coloboma, Heart defect, Atresia choanae, Retarded growth and development, Genital hypoplasia, Ear anomalies/deafness syndrome

CNV	copy number variants
COS7	CV-1 in Origin with SV40 genes
dB	decibel
DFN	DeaFNess
DFNA	nonsyndromic deafness, autosomal dominant
DFNB	nonsyndromic deafness autosomal recessive
DFNX	nonsyndromic deafness, X- linked
DNA	deoxyribonucleic acid
EVA	Enlarged vestibular aqueduct
FDA	Food and Drug Administration
HARS	histidyl tRNA synthetase
HGP	Human Genome Project
HL	hearing loss
GATK	Genome Analysis Toolkit
IHC	inner hair cell
JLNS	Jervell and Lange-Nielsen syndrome
MPS	massive parallel sequencing
mRNA	messenger RNA
NGS	next generation sequencing
NIDCD	National Institute on Deafness and Other Communication Disorders
NSHL	non-syndromic hearing loss
OHC	outer hair cell
OMIM	Online Mendelian Inheritance in Man
PCR	polymerase chain reaction
PRLTS1	Perrault syndrome 1
QTc	Corrected QT Interval
SHL	syndromic hearing loss
SNHL	sensorineural hearing loss
SNP	single nucleotide polymorphism
SNV	single nucleotide variant
STL1	Type I Stickler syndrome
UCSC	University of California, Santa Cruz

USH1	2, 3, Usher syndrome 1, 2, 3
WES	whole exome sequencing
WGS	whole genome sequencing
WHO	World Health Organization
WS1	2, 3, 4, Waardenburg syndrome 1, 2, 3, 4

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Key Points

1. Syndromic hearing loss (SHL) is a form of hearing loss (HL) accompanied by additional clinical features in the visual, nervous system, endocrine and other systems. The most prevalent syndromes are Usher, Waardenburg and Pendred.
2. Genetic diagnostics can detect pathogenic variants and provide an answer regarding the cause of the HL, as well as the associated clinical symptoms of the SHL, to care for the patient.
3. Linkage analysis with DNA markers and PCR diagnostics is often used to detect these variants in clinical settings. High-throughput sequencing methods, focusing on either specific genes, the exons of genes or the entire genome of a patient, are moving into the clinic to provide more cost effective and efficient methods for diagnostics.

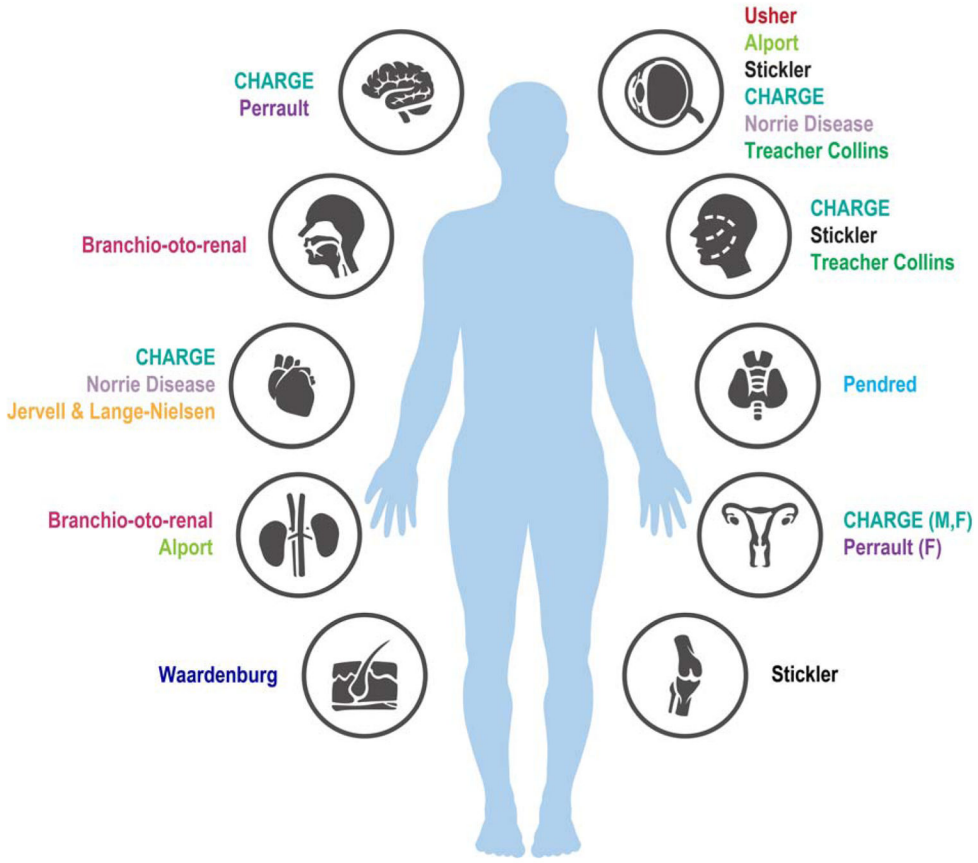

Fig. 1.

Different organs are involved in the clinical symptoms of patients with SHL, in addition to the phenotype in the inner ear. The organs affected in each syndrome are indicated. F, female genitals; M, male genitals.

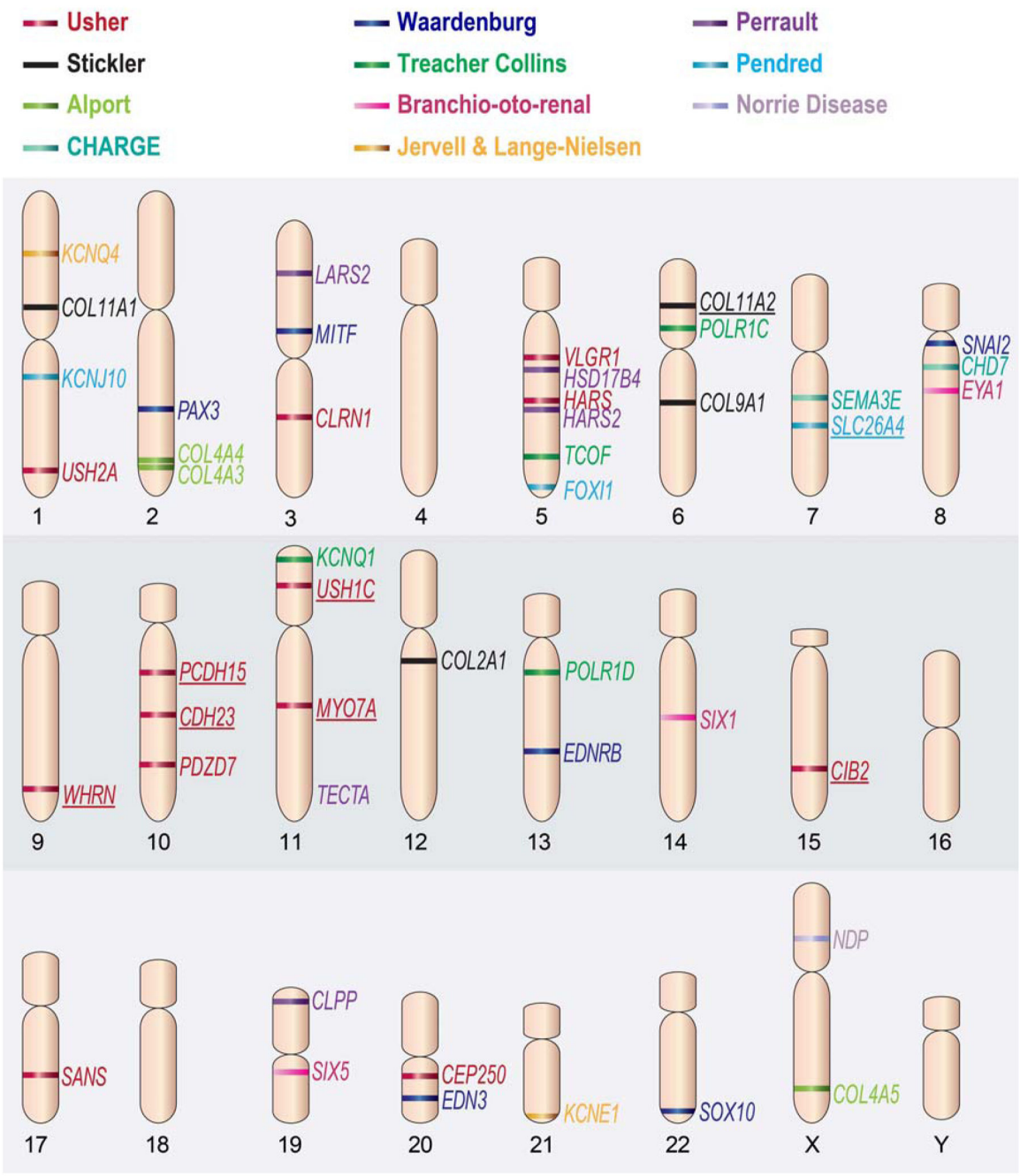

Fig. 2.

Schematic representation of the chromosomal location of genes associated with SHL. The genes are color coded according to the syndrome they are associated with. The genes associated with both syndromic and non-syndromic hearing loss are underlined. *Adapted from* Dror AA, Avraham KB. Hearing impairment: a panoply of genes and functions. Neuron. 2010;68:293–308; with permission.

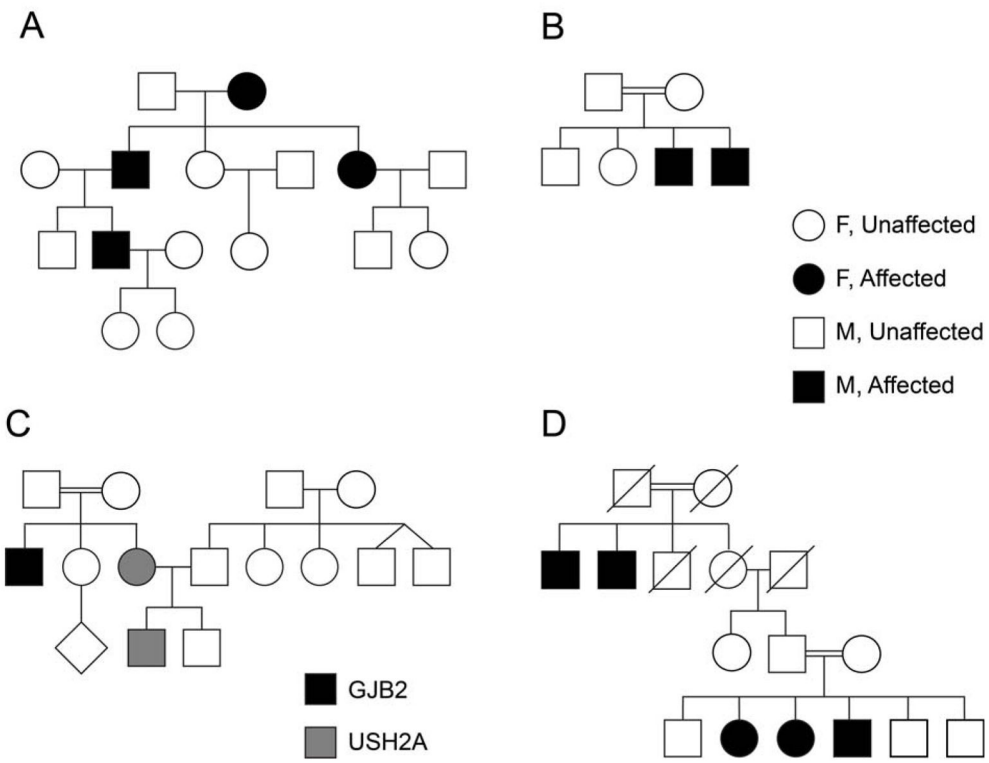

Fig. 3.

Representative pedigrees of families with HL. (A) Recessive inheritance with no previous family history. After biallelic variants were found in the child, the parents were each found to be carriers of the variant, validating the recessive pattern of inheritance. (B) Recessive inheritance with family history. The family represented in the pedigree presented with symptoms of USH1, with five affected individuals. The chromosomal critical region was defined by the use of microsatellite DNA markers, pinpointing the locus to chromosome 15q22[86]. Several years later, *CIB2* was found to be the pathogenic variant responsible for this new form of Usher syndrome, USH1J[32]. (C) Dominant inheritance, with an affected individual in three out of four generations, which may be observed for Waardenburg syndrome with a *PAX3, SNAI2* or *MITF* mutation. (D) A family with both SHL and NSHL. Two patients have Usher syndrome type II, with pathogenic variants in the *USH2A* gene. One patient has NSHL, due to biallelic pathogenic variants in the *GJB2* gene[111].

Data from Refs[32, 86, 111]

Table 1

Informative websites for SHL

WHO	http://www.who.int/topics/deafness/en/
ASHA	http://www.asha.org/public/hearing/Degree-of-Hearing-Loss/)
NIDCD	http://www.nidcd.nih.gov/health/statistics/Pages/quick.aspx
Hereditary Hearing Loss Homepage	http://hereditaryhearingloss.org/
Deafness Variation Database	http://deafnessvariationdatabase.org/
Leiden Open Variation Database	http://grenada.lumc.nl/LOVD2/WS/
Genetics Home Reference	http://ghr.nlm.nih.gov/
Online Mendelian Inheritance in Man	http://www.ncbi.nlm.nih.gov/omim/
UCSC Genome Browser	https://genome.ucsc.edu/
Genome Reference Consortium	http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/
GATK	https://www.broadinstitute.org/gatk/
GenVec	http://www.genvec.com/product-pipeline/cgf-166-hearing-loss

Table 2

Syndromic hearing loss-associated genes

Syndrome	Gene	Protein	OMIM[1] entry
Alport Syndrome	*COL4A5*	Collagen, type IV, alpha 5	303630
	COL4A3	Collagen, type IV, alpha 3	120070
	COL4A4	Collagen, type IV, alpha 4	120131
Branchio-oto-renal Syndrome	*EYA1*	Eyes absent homolog 1	601653
	SIX5	Homeobox protein SIX5	600963
	SIX1	Homeobox protein SIX1	601205
CHARGE Syndrome	*SEMA3E*	Semaphorin 3E	608166
	CHD7	Chromodomain-helicase-DNA-binding protein 7	608892
Jervell & Lange-Nielsen Syndrome	*KCNQ1*	Potassium channel, voltage gated KQT-like subfamily Q, member 1	607542
	KCNE1	Potassium channel, voltage gated subfamily E regulatory beta subunit 1	176261
Norrie Disease	*NDP*	Norrie disease protein	300658
Pendred Syndrome	*SLC26A4*	Pendrin	605646
	FOXI1	Forkhead box protein I1 ATP-sensitive inward rectifier	601093
	KCNJ10	potassium channel 10	602208
Stickler Syndrome	*COL2A1*	Collagen, type II, alpha-1	120140
	COL11A1	Collagen, type XI, alpha-1	120280
	COL11A2	Collagen, type XI, alpha-2	120290
	COL9A1	Collagen, type IX, alpha-1	120210
	COL9A2	Collagen, type IX, alpha-2	120260
Treacher Collins Syndrome	*TCOF1*	Treacher Collins-Franceschetti syndrome 1	606847
	POLR1D	Polymerase I Polypeptide D	613715
	POLR1C	Polymerase I Polypeptide C	610060
Usher Syndrome	*MYO7A*	Myosin VIIA	276903
	USH1C	Harmonin	605242
	CDH23	Cadherin 23	605516
	PCDH15	Protocadherin 15	605514
	SANS	Scaffold protein containing ankyrin repeats and sam domain	607696
	CIB2	Calcium and integrin binding protein 2	605564
	USH2A	Usherin	608400
	VLGR1	Very large G-coupled protein receptor isoform b	602851
	WHRN	Whirlin	607928
	CLRN1	Clarin 1	606397
	HARS	Histidyl tRNA synthetase	142810
	PZDZ7	PDZ domain containing 7	NA[2]
	CEP250	250	NA

Syndrome	Gene	Protein	OMIM[1] entry
Waardenburg Syndrome	*PAX3*	Paired box 3	606567
	SNAI2	Snail homolog 2	602150
	EDN3	Endothelin 3	131242
	EDNRB	Endothelin receptor type B	131244
	MITF	Microphthalmia-associated transcription factor	156845
	SOX10	SRY box10	602229
Perrault Syndrome	*HSD17B4*	Hydroxysteroid (17-beta) dehydrogenase 4	601860
	HARS2	Histidyl-TRNA Synthetase 2	600783
	CLPP	Caseinolytic mitochondrial matrix peptidase proteolytic subunit	601119
	LARS2	Leucyl-tRNA synthetase2	604544

[1]OMIM: Online Mendelian Inheritance in Man

[2]NA: Not available