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Genetic Basis of Hearing Loss

Agnieszka Pollak and Monika Ołdak

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Abstract

Etiology of hearing impairment (HI) is complex and comprises genetic and environmental factors. Currently, the background of genetic hearing impairment is an area of intensive research and we are witnessing fast progress in this field. The story has begun in 1997 when the DFNB1 locus was discovered with *GJB*2 and *GJB*6 genes causative for almost 50% of cases of recessive, profound, prelingual hearing loss. Nowadays, we have much more possibilities for dissecting the reason of HI, but proper assessment of clinical symptoms is essential for selecting the most optimal diagnostic pathway. In the first stage, the detailed characteristic of hearing loss including its level established by pure tone audiometry (PTA) or auditory brainstem responses (ABR), age of onset, and other helpful features as progressive or no progressive type should be provided. Subsequently, the presence or absence of accompanying symptoms should be established and followed by a detailed analysis of pedigree. In addition, modern assistive algorithms such as AudioGene, Face2Gene, and POSSUM are also discussed. Taking into account the variety of causative genes and pathogenic variants underling hearing loss, searching for causative genes, after exclusion of the DFNB1 variants, should be performed with multigenic panels based on next-generation sequencing technology.

Keywords: gene, pathogenic variant, phenotype, diagnostic, pedigree, next-generation sequencing

1. Introduction

The dynamic development of new DNA sequencing technologies in recent years has given us unprecedented insight into the information encoded in the human genome. Introduction of these techniques into a clinical practice has put the diagnosis of genetic disorders to a much more advanced level with a very high detection rate of pathogenic variants. Hearing loss (HL) has also greatly benefited from the technological revolution as it is a genetically

heterogeneous condition with more than 100 different genes being involved in its pathogenesis, and novel genes are still being discovered (www.herediataryhearingloss.org; accessed 10/2017). As compared to the strategy of sequential analysis of single genes, the application of high-throughput DNA sequencing has increased the diagnostic yield of genetic causes of HL by approximately four times [1].

At the same time, the technological advancements have brought us to a higher level of complexity. Searching for HL-causing variants, often hundreds of genes have to be analyzed and we are flooded by huge amounts of information that are difficult to interpret [2]. It is partially overcome by still-improving computational tools and growing data from population studies, but an indispensable part of better planning of genetic testing and understanding its results is the information gathered from a thorough clinical examination and family history. Sometimes, the primary clinical data collected prior to genetic testing do not completely match the phenotypic features that could be expected from molecular findings. In such cases, clinical reevaluation is needed to better delineate the phenotype and verify whether the identified genetic variants are indeed responsible for the observed clinical features [3, 4].

2. Pedigree construction and analysis

The fundamental and basic element of genetic evaluation in the consultation room is the creation of a precise and accurate family pedigree based on the detailed medical interview. A genetic pedigree is a diagram of genetic relationship enriched with an information about health history, which allows to easily trace the transmission of symptoms, estimate whether symptoms may be caused by genetic reason, and evaluate the risk for other family members (including unborn) of an inherited disorder. Although in the archival literature, readers may encounter many different systems of constructing pedigrees [5], the current, uniform, and precise guidelines in this area should be followed and applied in both clinical practice and publications. Taking into account the dynamic rise of genetic knowledge within the last years, triggered by new technologies such as next-generation sequencing (NGS), clinicians and scientists should be familiar with preparation and interpretation of a pedigree. This allows a proper interpretation of medical and genetic information of the studied family. At the time of writing, an authority dealing with the standardization of the terminology used to describe a pedigree is the National Society of Genetic Counselors (NSGC, http://www.nsgc.org) established in 1979 in the United States of America (USA), with its two official journals: "Journal of Genetic Counseling" and "Perspectives in Genetic Counseling." The Pedigree Standardization Work Group (PSWG) operating within the NSGC established unified recommendations for standardized human pedigree in 1995 [6, 7], and updated it in 2008 [8]. Regarding the fact that no alternative comprehensive, analogous recommendations have been proposed, along with lack of critical comments on the proposed system, the PSWG established rules have become an accepted and international symbolic language of human genetic clinicians and researchers.

The most commonly used pedigree symbols, definitions, and abbreviations in compliance with the PSWG revised recommendations are presented in **Figure 1**.

With the symbols listed in **Figure 1**, a family tree should be created according to the following rules:

- At the beginning, a shaded symbol (square for male, circle for female) denoting the proband (an affected person from a family who came for a medical consultation as first) should be placed. In the bottom left corner, an arrow and letter "P" should be placed to uniquely mark that this individual is the proband.

- Above the symbols for proband's, ancestors starting with parents should be placed. The symbol denoting male partner should be placed to the left of the female's one if possible. Furthermore, symbols denoting both parents should be connected by a horizontal line (relationship line) and doubled if the parents are consanguineous. The line of descent should be placed in the middle of the horizontal line, which connects parents with the offspring (sibship line).

- All symbols representing siblings should be placed in the birth order starting from the left side, at the same height as the proband's symbol. Vertical lines (individual's line), linking symbols to the horizontal line (sibship line) above the symbols, must be placed for all siblings' symbols.

- According to the above-described rules, symbols representing all remaining family members should be placed and joined with the appropriate lines.

- Some additional, useful information such as disease, age, age at death, initials, or the first name may be placed below appropriate symbols.

The pedigree line definitions and rules of placing them within the pedigree are presented in **Figure 2**.

Additional most common symbols, rules, and family situations are gathered in **Figure 3**.

Since drawing pedigrees, especially for large families, is complicated and time consuming, it is worth to use computer programs that facilitate and accelerate this task. There are many professional and public tools, which are useful in the process of creating accurate family diagrams for both, clinical and educational purposes, e.g., Genial Pedigree Draw (http://www.pedigreedraw.com/), Progeny Online Pedigree Tool (http://www.progenygenetics.com/online-pedigree/), and CeGaT Pedigree Chart Designer (http://www.cegat.de/en/for-physicians/pedigree-chart-designer/).

Understanding the elementary rules of inheritance is the key to appreciate how traits or diseases are passed on within a family. It should be reminded here that every individual has two copies of almost every gene localized on chromosome (autosome), one of them derives from biological mother and the second one from biological father. The situation is different in case of sex chromosomes—every male has only one X (inherited from mother) and Y chromosome (inherited from father). The Y chromosome is transmitted in its entirety exclusively from
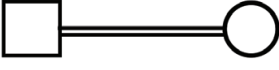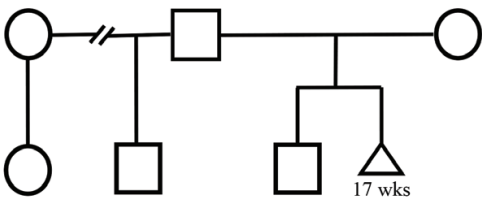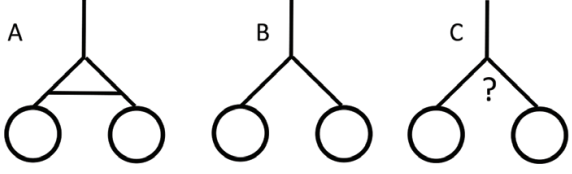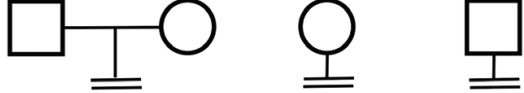
	Female	Male	Gender unknown	Remarks
Healthy person	○	□	◇	The age of the individuals should be placed below the symbol.
Affected person	●	■	◆	The symbols may be partioned and filled with different pattern if ≥ two symptoms occurred. The used patterns must be defined in the legend.
Deceased person	⌀	⬛̸	◇̸	The age at death should be placed below the symbol. If the cause of death is known it also should be indicated.
Proband	P↗●	P↗■		A first affected individual coming to the consultation.
Consultand	↗○	↗□		The person(s) referred for genetic counseling.
Multiple persons, number known	③	③	◇3	If the number is unknown or unstated the letter „n" should be used instead of a number.
Pregnancy	Ⓟ	□P	◇P	For affected individuals light shading should be used. Gestational age and karyotype should be denoted below the symbols.
Spontaneous abortion			△	Gestational age, gender should be denoted below the symbols
Termination of pregnancy			⟁̸	Gestational age, gender should be denoted below the symbols
Ectopic pregnancy			⟁̸ ECT	ECT should be given below the symbols

Figure 1. Most common pedigree symbols according to Bennett et al. [8].

Figure 2. Definition of pedigree lines.

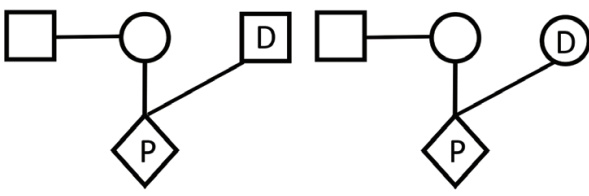| Definition	Symbols	Remarks
Consanguinity		Relationship which is not evident regarding pedigree should be described above relationship line
Relationship	17 wks	A gap within relationship line denotes that relationship currently is not continued
Multiple gestation	A B C ?	A. Monozygotic B. Dizygotic C. Unknown
Infertility		If the reason is known, should be indicated below the symbol
Adoption	A B	Biological parents are denoted by solid line, adoptive parents are denoted by dashed line. A. In B. Out
Carrier		The disease is not manifested
Asymptomatic or presymptomatic carrier		Individual without clinical symptoms at the time of completing the medical history
Sperm/ovum donor	D D P P	The line of descent is solid denoting biologic relationship which may have influence to the fetus

Figure 3. Symbols useful in uncommon clinical situations.

father to son. In contrast, every female has two X chromosomes (inherited from both parents) [9]. Another derogation from basic inheritance rules is mitochondrial inheritance, in which the entire independent small genome is passed only from mother to offspring [10]. Typically,

there are four most common inheritance patterns, depending on the genomic localization and influence on the protein function of pathogenic variants or genes i.e., autosomal dominant (AD), autosomal recessive (AR), sex-linked, and mitochondrial [11]. Diseases caused by pathogenic variants localized in a single gene are mostly inherited in an AD or AR pattern and are referred to as Mendelian inheritance (tribute to Gregor Mendel, who first noted this pattern in pea plants).

The AD mode of inheritance occurs when a single copy of the disrupted (mutated) gene is causative of the disease. It should be emphasized that for AD disorders, a variety of inter and intrafamilial variability of symptoms may occur. Nevertheless, there are few substantial features, which make this mode of inheritance rather simple to distinguish. Dominantly inherited genetic diseases tend to occur in every generation of a family, they affect males and females equally; furthermore, the disorder may be transmitted from males and females. The risk for offspring to inherit the pathogenic variant is 50%. Due to the variability of symptoms severity, characteristic for this type of inheritance, the risk of becoming symptomatic may be less than 50%. A typical family tree representing the AD mode of inheritance is shown in **Figure 4**.

The AR type of inheritance requires two disrupted copies of a gene for a disease to occur. Thus, both parents of an affected individual are obligatory asymptomatic carriers (due to an assumption that heterozygotes do not manifest a disease). Furthermore, the symptoms are not typically seen in every generation. AR diseases are much more common in offspring of consanguineous pairs (which is evident within small, isolated populations e.g., Icelanders, Bedouins, or Amish [12]. In contrast to the AD inheritance mode, affected individuals present more consistent clinical picture. The risk for offspring to inherit both pathogenic variants is 25%, whereas the risk to inherit one heterozygous variant is 50%. It should be also noted that all children of an affected parent and a noncarrier partner, regardless of their gender, will be obligate carriers. A typical family pedigree illustrating the AR mode of inheritance is shown in **Figure 5**.

A sex-linked mode of inheritance consists of three subtypes: X-linked dominant (XLD), X-linked recessive (XLR), and Y-linked. Whereas the Y chromosome contains very limited
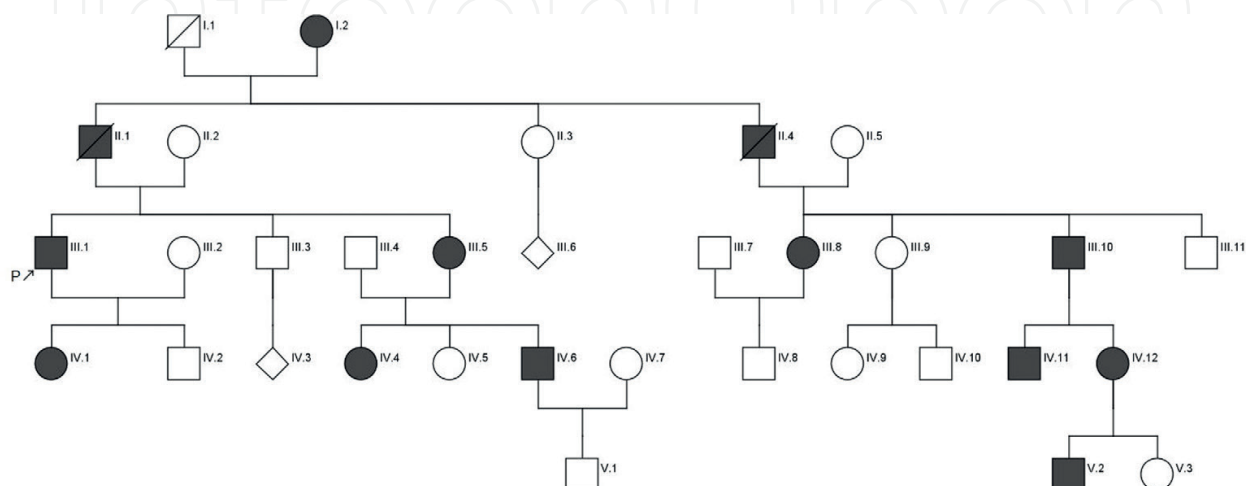

Figure 4. Pedigree of a family with the AD pattern of inheritance.
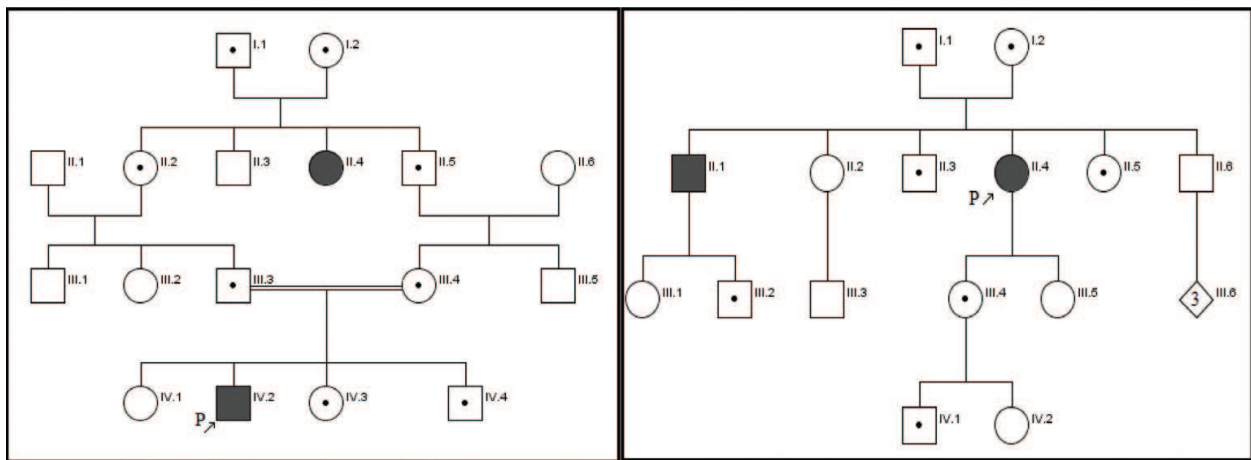
Figure 5. Two typical family trees representing the AR type of inheritance.

number of genes and there are only few Y-linked disorders, none of them related to hearing and speech disorders, this type of inheritance will not be described here. The XLD and XLR type of inheritance relate to genes located on the X chromosome, and for the occurrence of XLD symptoms, only one copy of a disrupted X-linked gene is required. XLD diseases usually manifest very severely in males, which may lead to spontaneous abortion or neonatal death. The characteristic feature for this type of inheritance is that there is no transmission of the disease from male to male, and all of the female offspring of affected male will inherit the pathogenic variant and the disease. A characteristic family tree for the XLD mode of inheritance is shown in **Figure 6**.
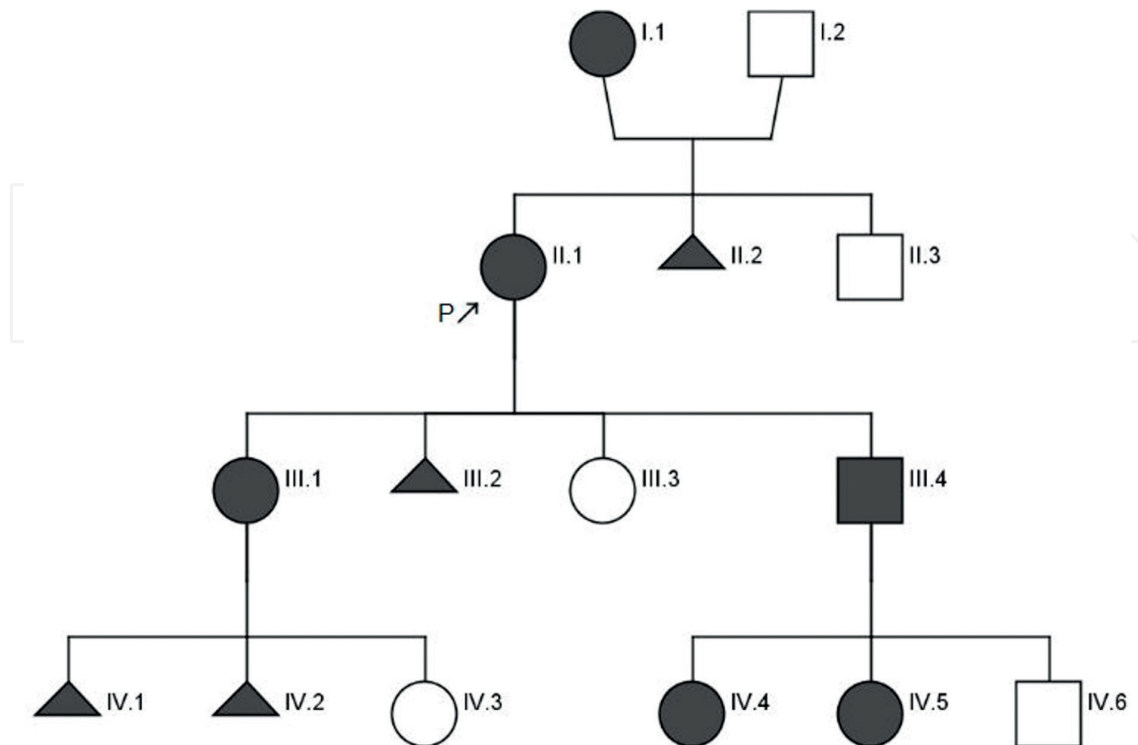

Figure 6. Exemplary pedigree of a family with the XLD type of inheritance.

For an XLR disease to occur in females, both copies of a gene must be impaired. Characteristic features for an XLR inheritance mode are affected males, but an extremely low number or no affected females, in every generation. A distinct feature of the XLR inheritance pattern is that the pedigree tree shows no male to male transmission of the disease. All males harboring a pathogenic variant in an X-linked gene present severe symptoms of the disease, whereas carrier females are in general unaffected or present significantly less severe symptoms. A typical family tree representing the XLR mode of inheritance is shown in **Figure 7**.

Although within small families, which currently are very common, especially in European countries, the recognition of an X-linked inheritance pattern is rather challenging and remains unknown until the results of genetic testing [4].

The mitochondrial mode of inheritance has distinctive features differentiating it from others. Briefly, this unique features come directly from mitochondrial DNA (mtDNA) specificity: mtDNA is a small, independent, circular genome; furthermore, an average human cell contains up to 1000 mitochondria and in every mitochondrion several copies of the mtDNA genome may be present. If all mitochondria in a given individual contain an mtDNA variant it is defined as homoplasmy. In contrast, heteroplasmy indicates the coexistence of more than one mtDNA type within an individual. As all mitochondria of offspring are of maternal origin, the pathogenic variants localized within mtDNA are exclusively passed from mother to children and they may affect males and females equally. Consistently, males do not transmit the mtDNA disorders to their offspring. A representative family tree demonstrating mitochondrial mode of inheritance is shown in **Figure 8**.
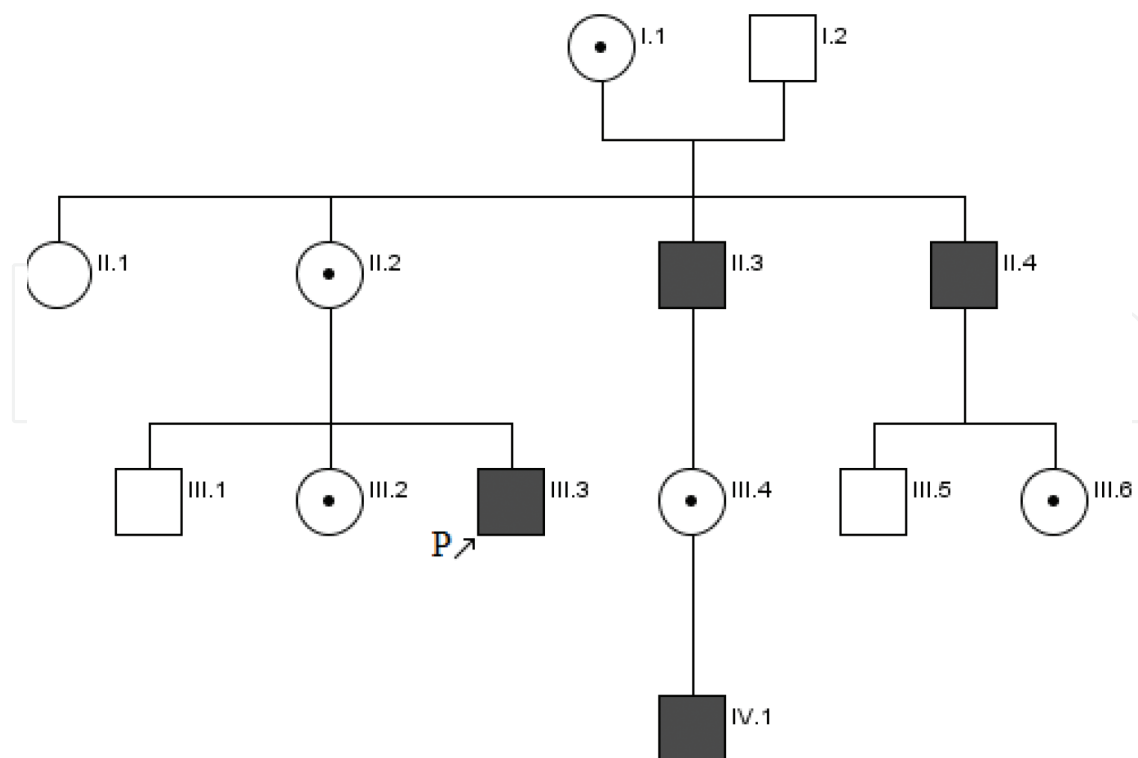

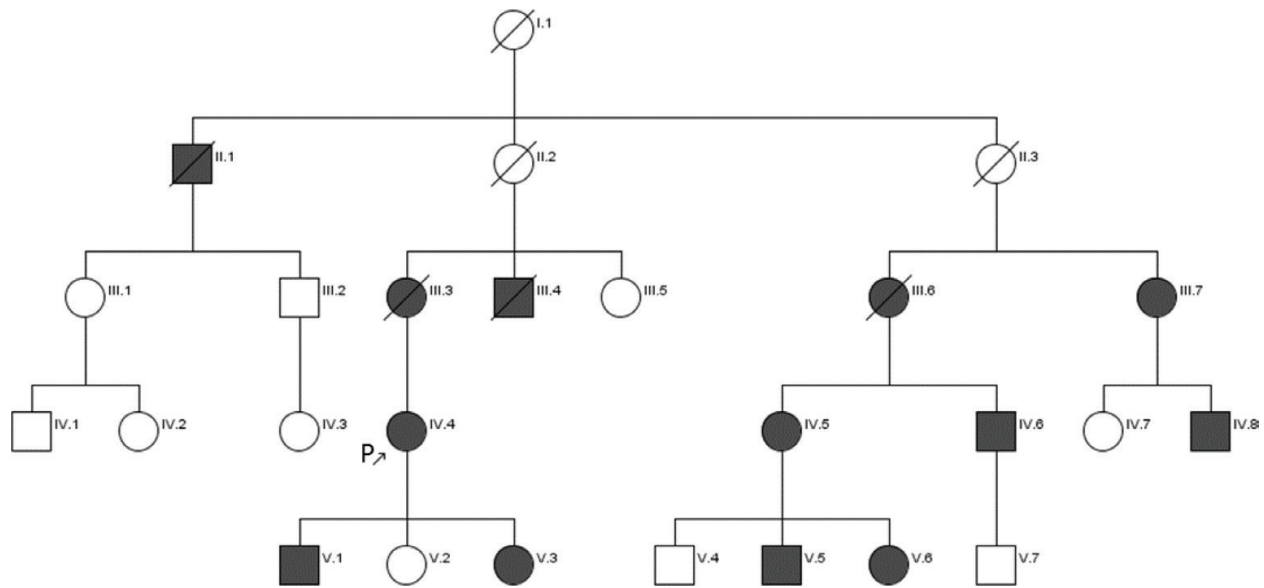**Figure 7.** Pedigree of a family with the XLR pattern of inheritance.

Figure 8. Pedigree of a family with mitochondrial mode of inheritance.

It should be emphasized that in many cases distinction between the mitochondrial and AD inheritance is a difficult task based only on the family tree analysis.

3. Nonsyndromic hearing loss

It has to be stressed that all patients with a positive family history of HL should be referred for genetic counseling. Over generations, an inherited disorder automatically raises a suspicion of a genetic underlying cause. A careful analysis of a family pedigree enables to identify or to presume the mode in which hearing impairment is inherited. This in turn is an important step in directing genetic testing at specific genes that are causally involved in the pathogenesis of autosomal dominant, recessive, X-linked, or mitochondrial HL. In case of marriages between individuals with hearing impairment or between individuals coming from families with hearing impairment, the family pedigrees should be analyzed especially carefully. In the offspring of such couples, different HL causative variants of different genes may be found. Interestingly, in a study of 80 deafness genes, the DNA samples of HL patients were significantly enriched in potentially pathogenic variants [13]. One possible explanation of the phenomenon may the above-mentioned marriages between hearing impaired individuals. Attention should also be paid to the consanguinity between parents, which is common in certain populations. Looking for a genetic cause of HL in offspring of a consanguineous couple, the autosomal recessive mode of inheritance with pathogenic variants in a homozygous state is primarily expected.

A diagnostic challenge represents an HL patient without other affected family members, also referred to as a sporadic case. Here, a family history of HL is negative and a genetic cause is strongly suspected after exclusion of environmental factors, such as prenatal infection (with toxoplasmosis, rubella, cytomegalovirus, and herpesvirus—"TORCH" organisms), postnatal infections (mainly bacterial meningitis, mumps), prematurity, traumatic injury, blood vessels

or autoimmune disease, Meniere's disease, acoustic neuroma, exposure to chemical agents, or noise that may be responsible for HL development. While describing HL, four major terms related to its presentation such as (I) the age of onset, (II) the type, (III) the degree of HL, and (IV) stability are usually used. The onset of HL can be congenital, prelingual (before a child develops speech), postlingual (after the acquisition of speech and language, usually after the age of six), adult-onset or age-related late-onset (presbyacusis). The different types of HL (conductive, sensorineural, or mixed) indicate which part of the ear is affected. Genetically determined HL is usually bilateral although families with asymmetric and unilateral HL are also reported [14, 15].

It has been estimated that about 80% of prelingual HL results from genetic factors. It is most often inherited as an autosomal recessive feature without other accompanying medical problems The second most common inheritance pattern of prelingual HL is autosomal dominant (20%), while X-linked and mitochondrial constitute together approximately 1–1.5% [16]. Most of the reported families with nonsyndromic postlingual HL present an autosomal dominant pattern of inheritance. Currently, 36 different genes causally involved in autosomal dominant HL have been identified (www.herediataryhearingloss.org; accessed 10/2017) and only a few of them are associated with prelingual HL [17].

If hearing impairment represents an isolated finding that can be associated with abnormalities of the middle and/or inner ear but is not accompanied by visible abnormalities of the outer ear or any other medical problems, it is referred to as nonsyndromic or isolated. The major cause of prelingual severe-to-profound autosomal recessive nonsyndromic HL in many populations are pathogenic variants of the *GJB2* gene. The *GJB2* and *GJB6* genes, contained within the *DFNB1* locus, should be tested in the first line in patients with nonsyndromic bilateral sensorineural HL of the prelingual onset [18].

Pathogenic *GJB2* variants are also identified as the second most frequent cause of mild-to-moderate autosomal recessive HL. The most common causes of HL in this group of patients are pathogenic variants of the *STRC* gene and the third causative gene in this category is *TECTA*, but the prevalences vary among different ethnic groups [13, 19].

Discussing the genetic causes underlying partial deafness, defined as normal or slightly deteriorated thresholds involving low frequencies combined with profound HL in high frequencies [20], pathogenic variants localized within mtDNA and *TMPRSS3* should be considered for diagnostic purposes [21, 22]. Nevertheless, the contribution of other genes should be also taken into account.

Inheritance pattern	Genes and loci involved
AD	*COCH* (DFNA9), *WFS1* (DFNA6/14/38), *MYO6* (DFNA22), *GJB2* (DFN3A), *MYO7A* (DFNA11)
AR	*GJB2* (DFNB1A), *SLC26A4* (DFNB4), *MYO15A* (DFNB3), *TMC1* (DFNB7/11), *TMPRSS3* (DFNB8/10), *STRC* (DFNB16)
X-linked	*POU3F4* (DFNX2), *PRPS1* (DFNX1), *SMPX* (DFNX4), *COL4A6* (DFNX6), *AIFM1* (DFNX5)
Mitochondrial	*MT-TL1*, *MT-TK*, *MT-TS1*, *MT-TE*, *MT-RNR1*

Table 1. Genes involved in the pathogenesis of hearing disorders grouped according to the type of inheritance—examples.
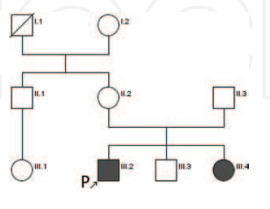
Locus DFNB1, gene *GJB*2, inheritance type: AR

Remarks

Truncating pathogenic
variants (e.g., c.35delG)
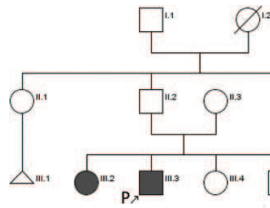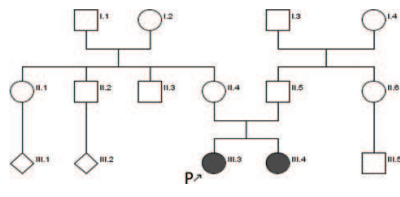

Congenital, bilateral, profound
HL [23]

Nontruncating pathogenic
variants (e.g., p.Met34Thr)

Postlingual, bilateral, mild-to-
moderate HL [24]

Locus DFNB4, gene *SLC*26*A*4, inheritance type: AR

Remarks
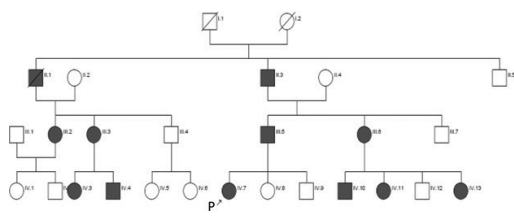
Nontruncating,
pathogenic variants (e.g.,
p.Glu29Gln)

Enlarged vestibular aqueduct,
vestibular dysfunction, Mondini
malformation, early-onset,
fluctuating HL [25]

Locus DFNA6/14/38, gene *WFS*1, inheritance type: AD

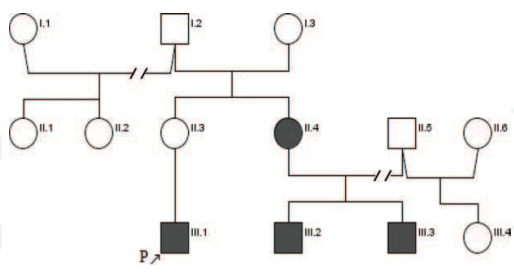**Remarks**

Nontruncating,
pathogenic variants (e.g.,
p.Asp771His)

Postlingual, low-frequency HL,
deteriorating with time [26]

Locus DFNX2, gene *POU*3*F*4, inheritance type: X-linked

Remarks

Truncating pathogenic
variants (e.g., p.Glu187*)

Congenital, profound, sensorineural
HL (may be accompanied by a
conductive component). Inner ear
IP3 type malformation—comprises
of enlarged internal auditory
canal and vestibular aqueduct,
underdeveloped

cochlear modiolus and
malformations of the vestibule.
Due to the inner ear malformation
perilymphatic gusher may occur
during inner ear surgery [4]

Gene *MT-TS*1, inheritance type: X-linked

Remarks

Pathogenic variants (e.g.,
m.7511T>C)
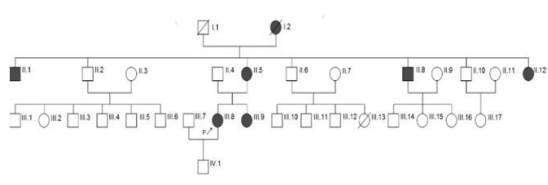
Postlingual, high-frequency HL [27]

Table 2. Pedigrees and audiometric features characteristic for different genes and pathogenic variants causative of HL.

Considering the significant contribution of genetic factors to HL and the recent guideline for clinical evaluation and etiologic diagnosis of HL, one may conclude that single-gene testing is justified if a specific genetic etiology of HL is suspected. If there are no specific clinical indications, testing for the DFNB1-related HL should be performed. If the investigations do not provide conclusive results, HL genes may be analyzed by the NGS approaches such as multigene panels, whole exome or whole genome sequencing [16]. All known nonsyndromic deafness loci (locus denotes the position in the genome linked with the disease) are labeled as DFN (DeaFNess) and classified according to the type of inheritance (DFNA: autosomal dominant; DFNB: autosomal recessive; DFNX: X-linked) followed by a number indicating the order of locus discovery. In **Table 1**, some of the most common HL causative loci are gathered.

As it was previously stated, HL is a genetically heterogenous disease; nevertheless, there are some common, characteristic features, which may be a valuable asset in the process of dissecting the genetic reason of HL. Examples of pedigrees, characteristic audiometric features, and additional remarks for some common HL genes are shown in **Table 2**.

Despite quite a large number of genes causative for HL, the first step in the diagnostic approach should be the analysis of the DFNB1 locus, as the testing is inexpensive and fast [28]. Apart from obvious clinical indications, such as IP3 malformation for the *POU3F*4 gene analysis, the remaining cases should be rather streamed to wide, multigenic analysis.

4. Syndromic hearing loss

In contrast to the nonsyndromic hearing impairment, the syndromic hearing impairment is defined as a part of a syndrome, and it is associated with malformations of the external ear, malformations of other organs or other medical problems. HL is identified in more than 400 different syndromes [29]. The most common type of autosomal dominant syndrome with sensorineural HL is **Waardenburg syndrome** (WS types 1-4; OMIM#193500, #193510, #148820, and #277580), an auditory-pigmentary syndrome caused by pathogenic variants in *PAX*3, *MITF*, *EDNRB*, *EDN*3, or *SOX*10 genes and characterized by the presence of pigmentary anomalies of skin, hair, and eyes. The second most common are **branchiootorenal spectrum disorders** (OMIM#113650, #610896 #602588, #166780), where conductive/sensorineural HL is accompanied by branchial cleft cysts or fistulas, malformations of outer ear, preauricular pits, and renal anomalies resulting from pathogenic variants in *EYA*1, *SIX*1, or *SIX*5. The third most common cause of autosomal dominant syndromic hearing impairment is **neurofibromatosis type 2** (NF2, OMIM#101000), a multiple neoplasia syndrome with HL secondary to the usually bilateral vestibular schwannomas (acoustic neuroma) and other tumors of the central nervous system (meningioma, schwannoma, glioma, or neurofibroma). NF2 is caused by heterozygous pathogenic variants in a gene-encoding neurofibromin-2 (NF2 gene) [30].

The **Stickler syndrome** (STL) is a connective tissue disorder with eye findings (high myopia, vitreoretinal degeneration, retinal detachment, and cataract) being the most constant traits. Other features are sensorineural or conductive HL, midline clefting (cleft palate, bifid uvula), Pierre Robin sequence, flat midface, mild spondyloephiphyseal dysplasia, and

early-onset osteoarthritis [31]. Currently, four types of STL syndrome are distinguished—autosomal dominant STL1 (OMIM#108300) and STL2 (OMIM# 604841) caused by pathogenic variants in *COL2A1* and *COL11A1*, and autosomal recessive STL4 (OMIM#614134) and STL5 (OMIM#614284) due to pathogenic variants in *COL9A1* and *COL9A2* genes, respectively. The **Usher syndrome** (USH) is a combination of HL and visual impairment as a consequence of retinitis pigmentosa. The autosomal recessive condition is classified into three types: USH1 (OMIM#276900) with severe-to-profound deafness and defective vestibular function, USH2 (OMIM#276901) with mild-to-severe hearing impairment and normal vestibular function and USH3 (OMIM#276902) with progressive postlingual HL and vestibular dysfunction. Pathogenic variants in one of six genes (*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, or *CIB2*) may lead to USH1 [32], in one of three genes (*ADGRV1*, *WHRN*, or *USH2A*) to USH2 and in one of two genes (*CLRN1* or *HARS*) to USH3. The second most common autosomal recessive syndrome with sensorineural HL is the **Pendred syndrome** (PDS, OMIM#274600), characterized by severe-to profound deafness that is congenital or develops in early childhood and euthyroid/hypothyroid goiter that arises in early puberty or adulthood. It is associated with developmental abnormalities of the cochlea (Mondini dysplasia or enlarged vestibular aqueduct) that can be diagnosed by a CT examination of temporal bones. The cause of the PDS is pathogenic variants in the *SLAC26A4* gene encoding an anion transporter named pendrin. The third most common autosomal recessive syndrome with deafness is the **Jervell and Lange-Nielsen syndrome** (JLN), which is marked by congenital profound sensorineural HL and prolongation of the QT interval (corrected QT (QTc) > 440 msec), syncopal episodes due to ventricular arrhythmias and a high risk of sudden death. In patients with JLNS1 (OMIM#220400) pathogenic variants in *KCNQ1* and in patients with JLNS2 (OMIM#612347) pathogenic variants in *KCNE1* are found.

The two following autosomal recessive syndromic forms of HL represent rare metabolic disorders that, however, should not be missed out as their symptoms may resolve by appropriate treatment and dietary modifications. The **Biotinidase deficiency** (BTD, OMIM#253260) is a form of multiple carboxylase deficiency characterized by primarily neurologic (seizures, hypertonia, developmental delay, ataxia) and cutaneous (skin rash, dermatitis, alopecia) features. Patients lose vision and three-fourth of those who become symptomatic have some degree of HL. Laboratory findings show organic aciduria, mild hyperammonemia, and biotinidase deficiency. The BTD begins usually within the two first years of life and results from recessive pathogenic variants in the *BTD* gene. Treatment with biotin resolves neurologic and cutaneous manifestations, while HL and optic atrophy are usually irreversible.

The **Refsum disease** (OMIM#266500) is an inborn error of lipid metabolism with anosmia and early-onset retinitis pigmentosa being two universal findings. Other variable clinical features include neuropathy, ataxia, progressive severe HL, ichthyosis, cardiac, and skeletal (metacarpals/metatarsals shortening) involvement. Increased serum concentration of phytanic acid establishes the diagnosis. The symptoms present an insidious onset usually during the late first through third decades of life. Causative recessive variants are found in the *PHYH* and *PEX7* genes. In the medical care, diet modifications aimed at reduction of chlorophyll from the diet (exclusion of green vegetables (phytanic acid), and animal fat (phytol), and plasmapheresis) are used.

Syndromic X-linked HL is represented by the **Alport** (OMIM#301050) and **Mohr-Tranebjaerg** (MTS, OMIM#304700) **syndromes**. The Alport syndrome is characterized by glomerulone-phropathy leading to progressive renal failure, varying severity of progressive sensorineural HL (occurs typically after 10 years of age and affects mainly high frequencies), and variable ocular anomalies. In 85% of patients with Alport syndrome, dominant pathogenic variants in the *COL4A*5 gene on the X chromosome are found. Approximately 15% of patients develop Alport syndrome due to recessive variants in the *COL4A*4 or *COL4A*3 genes, cases with auto-somal dominant inheritance, have also been occasionally reported.

In the Mohr-Tranebjaerg syndrome, also known as progressive deafness syndrome with blindness, dystonia, fractures and mental deficiency, progressive pre- or postlingual sensori-neural deafness occurs in the early childhood and is a presenting symptom. MTS is caused by recessive variants of the *TIMM*8A gene.

Mutations in the **mitochondrial genome** can lead to HL that is an isolated feature or part of genetic syndrome, i.e., mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERFF) or Kearns-Sayre syn-drome (KSS), a mitochondrial myopathy, characterized by ptosis, ophthalmoplegia, muscle weakness, cerebellar ataxia, diabetes mellitus, and/or endocrinopathies [33]. Aminoglycoside ototoxicity has been associated with pathogenic variants in *MT-RNR1* [34]. Pathogenic variant m.3243A > G in the *MT-TL1* gene, which is causative of MELAS, was found in patients with diabetes mellitus and HL or HL exclusively [35]. The data raise questions on mitochondrial variant penetrance, tissue specificity, and heteroplasmy level.

5. Assistive algorithms

Regarding the heterogeneity of genetically related HL, the precise evaluation of its cause is a challenging task. Apart from some, rather rare, unquestionable phenotypic manifestations, many of the cases of possibly genetically related HL (syndromic and nonsyndromic) are hard to distinguish. It should be noted that there is a group of useful, assistive algorithms, which are helpful for both: establishing the diagnosis and targeting a diagnostic approach.

The Face2Gene (http://suite.face2gene.com/) is a collection of phenotyping applications, which enable accurate and comprehensive assessment of a patient based on characteristic dysmor-phic facial features. As the variations in the face and skull are very common and are present in about 40% of genetic disorders, they are very useful in identification of the disorder. Briefly, the picture of patient face is uploaded to an application, than the quantification of the similarity is calculated with the usage of the algorithm database. The result is the list of syndromes with analogous morphology. The algorithm Face2Gene is accessible free of charge to all healthcare specialists.

The AudioGene (http://audiogene.eng.uiowa.edu/) predicts the genes underling autosomal dominant nonsyndromic hearing loss (ADNSHL) based on the audiometric data. This algo-rithm also takes into account the age of the patient, which is essential in the ADNSHL study

due to characteristic deterioration of hearing during patient's life. The algorithm is based on the computational analysis of specific audiometric data deposited in the database. Furthermore, the machine-learning method is applied to select and prioritize possibly causative genes for pathogenic variant screening [36, 37].

The Pictures of Standard Syndromes and Undiagnosed Malformations (POSSUM) (https://www.possum.net.au/) is a comprehensive dysmorphology database designed for the diagnosis of multiple malformations, metabolic, teratogenic, chromosomal, and skeletal syndromes as well as their images. This database consists of information on over 4000 different syndromes and searching is based on the selected features and results in a specific list of possible diagnoses [38].

6. Next-generation sequencing technology in dissecting the background of hearing loss

In almost all genetically related diseases (included hearing impairment), there is a need for tailored diagnostic strategies in searching for their molecular background. The milestone in this research area was the development of the method for the DNA sequencing by Frederick Sanger in 1975 [39]. Whereas the direct sequencing (called also Sanger sequencing—tribute to its inventor) allows to debunk the molecular cause of disease in a limited number of genes, e.g., when HL background analyses were limited to the *GJB*2 gene, there was a significant gap in our knowledge and diagnostic capabilities. This gap has been filled out by introducing NGS technology, also called massive parallel sequencing (MPS) or high-throughput DNA sequencing. The NGS developed over the last decade has revolutionized the genetic research and diagnostic practice, mainly because of reducing costs and time of DNA sequencing. This technology gives a unique opportunity to analyze in one experiment the sequence of more than 1 million base pairs, thus sequencing of the whole genome or thousands of genes simultaneously in a few days has become possible [40]. The most commonly used forms of NGS in searching for pathogenic variants underling HL are whole exome sequencing (WES) and multigene panels.

An undisputable advantage of the WES is the possibility of simultaneous sequencing of the whole coding DNA sequence (protein coding part within all human genes ~20,000) regardless of the disease studied. This makes WES a universal test for almost all known genetic diseases. The multigene panel sequencing is a more economical solution, especially dedicated for the diagnostic purpose. It should be noted that with the panel sequencing, only already known genes associated with a given disease are analyzed. Although the targeted NGS has many advantages, whole-genome sequencing (WGS) is still the state of art approach with its high cost representing a main drawback. The application of high-throughput DNA sequencing methods generates a vast amount of information, which accelerated the discovery of new, causative genes for many diseases. Also in the field of genetically related HL, with the NGS technology, novel genes have been discovered for all modes of inheritance described above. The important, newly discovered genes causative of recessive HL are: *ADCY*1, *BDP*1, *SYNE*4, *ELMOD*3, *CABP*2, *GRXCR*2, *OTOGL, TPRN*, and *TSPEAR*. For dominant HL, the *CEACAM*16, *P2RX*2, and *OSBPL*2 genes

were revealed, also gene for the DFNX4 locus (i.e., *SMPX*) was identified. Another remarkable achievement obtained by the NGS technology is the identification of genes incorrectly classified as pathogenic, the examples of such events are: *MYO*1*A* and *RAB*40*AL* [41–43].

For the clinical diagnostic purpose, there are many commercial tests based on NGS, which differ in technologies and numbers of genes included. Heretofore, at least 20 commercially available tests, based on the NGS technology, focused on genetically related HL may be applied [1]. Due to the constant reduction of costs and availability, diagnostic approach based on the NGS technology in the nearest future will become a standard, which will significantly improve the level of patient care.

Index of technical terms

Autosomal dominant inheritance (AD)—type of Mendelian inheritance of a trait in which a defective copy of a gene (localized on autosome) dominates over the normal one. For the symptoms to occur, presence of only one defective copy is sufficient.

Autosomal recessive inheritance (AR)—type of Mendelian inheritance of a trait in which two copies of defective gene (localized on autosomes) are required in order for the disease to develop.

*DFNB*1 **locus**—most common locus causative for nonsyndromic hearing loss, containing *GJB*2 and *GJB*6 genes.

Direct sequencing—a technology allowing to determine the sequence of nucleotides in DNA invented in 1977 by Frederic Sanger and Alan R. Coulson, based on the chain-dideoxy terminator method, also called Sanger sequencing.

Genetic pedigree—illustration of genetic relationship of a family, including information about health history of the family members.

Heteroplasmy—coexistence of more than one mtDNA type within an individual.

Homoplasmy—presence of a uniform type of mtDNA within an individual.

Mendelian inheritance—type of transmission of genes according to Gregor Mendel's set of laws, also called classical inheritance. Mendelian inheritance comprises of autosomal dominant, autosomal recessive, and X-linked type of inheritance.

Mitochondrial DNA (mtDNA)—small circular genome localized in the mitochondria.

Mitochondrial inheritance—non-Mendelian type of inheritance, occurring when a defective gene is located within the mitochondrial genome, inheritance of a trait encoded by this gene takes place exclusively from mother to offspring.

Next generation sequencing (NGS)—also known as high-throughput sequencing, a technology allowing to establish the sequence of DNA larger than 1 million base pairs in a single experiment.

Nonsyndromic deafness—deafness not associated with pathological symptoms/signs from other systems.

Partial deafness—hearing loss assessed by an audiometric test as a normal or little elevated hearing threshold within low frequencies and significantly raised hearing threshold in high frequencies.

Post-lingual hearing loss—hearing loss with late onset (after speech development).

Prelingual hearing loss—hearing loss with early onset (before speech development).

Sex-linked inheritance—type of the Mendelian inheritance of a trait in which the defective gene is located on X or Y chromosome.

Syndromic deafness—deafness as a part of a syndrome i.e., associated with pathological symptoms/signs from other organs.

Whole exome sequencing (WES)—approach based on NGS technology, allowing to sequence all protein-coding regions (exons).

Whole genome sequencing (WGS)—approach based on NGS technology allowing to analyze the whole genome DNA sequence.

Author details

Agnieszka Pollak* and Monika Ołdak

*Address all correspondence to: a.pollak@ifps.org.pl

Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland

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