Phenotypes of *SLC26A4* gene mutations – Pendred syndrome and hypoacusis with enlarged vestibular aqueduct

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Abstract

This paper presents the current views, regarding the pathomechanisms, which lead to the development of pathological symptoms in the enlargement of the vestibular aqueduct syndrome (EVAS) and the Pendred syndrome (PS). Associated phenotypes have been discussed and an attempt has been undertaken to correlate them with a corresponding genotype. Mutations of *SLC26A4* gene are one of the factors, which are at the base of congenital hearing losses. Inherited hearing loss occurs in these cases either as an isolated phenomenon with anatomical anomalies of the labyrinth in the background (EVAS) or with endocrine disorders (PS). The official name of *SLC26A4* gene is "solute carrier family 26, member 4". Pendrin, the product of its expression, transports iodine beyond thyroid follicular cells, where it is linked with thyroglobulin and, then, used in hormone synthesis. Abnormal expression of *SLC26A4* gene results in disturbance of iodine organification. In the internal ear, pendrin transports bicarbonates to the endolymph, taking in this way an active part in pH control of the endolymph and providing proper functioning of KCNJ10 potassium channels and TRP5 calcium channels. Disorders of homeostasis in labyrinth fluids are responsible for abnormalities of its structure, such as enlargement of the vestibular aqueduct and of the endolymph sac. At present, the Human Gene Mutations database provides 124 recessive mutations of *SLC26A4* gene. In EVAS and PS, two missense mutations are most frequently observed: L236P and T416P, as well as the mutation, regarding abnormal splicing process, i.e., IVS8+1G-A, in a total of 55% of the patients with recognised mutation of *SLC26A4* gene; the remaining 45% of changes of this gene are unique mutations.

REVIEW

INTRODUCTION

The Pendred syndrome (PS, MIM 274600) is an autosomally recessively inherited disease, which is caused by mutation in *SLC26A4* gene (MIM 605646). Its diagnosis requires identification of the classical triad of symptoms, including hypoacusis, thyroid goitre and iodine organification defect in the thyroid, which may lead to hypothyroidism.

The familial occurring, isolated hypoacusis with enlarged vestibular aqueduct, was for the first time described in 1996 (*DFNB4* gene, MIM 600791) [7] and has since then been confirmed in a number of reports. It is also referred to as the enlarged vestibular aqueduct syndrome – EVAS (MIM 603545) or the pseudo-Pendred syndrome [12, 16, 34]. EVAS, in its clinical picture, involves bilateral receiving sensorineural hearing loss, up to deafness, as well as labyrinth structure disorders.

GENETIC BACKGROUND

SLC26A4 gene

Exactly one hundred years after the description of a disease by Vaughan Pendred in 1896 [29], referred to since then by his name as the Pendred syndrome, a region was identified on the long arm of chromosome 7 (7q22.3-q31.1), within an interval of 9-cM (centimorgan), between GATA 23F05 and D7S687 loci, the genetic changes of which were related to occurrence of the syndrome in question [7, 41]. A year later, Everett et al. [9] identified *SLC26A4* (*PDS, DFNB4*, MIM 605646) gene, the mutations of which were responsible for PS and EVAS. The official name of this gene is "solute carrier family 26, member 4". This gene has got 21 exons and an open reading frame of 2343 bp in size. Messenger RNA specific for this gene is built of 4927 bp [39] (Fig. 1).

Product of SLC26A4 gene expression

The product of *SLC26A4* gene expression is pendrin – a 780 amino acid protein with molecular mass of 85 722 Da [22]. This protein is an integral part of the cellular membrane, and its polypeptide chain is 12 times anchored in the cellular membrane, passing through its structure from one side onto the other. This configuration means the presence of 12 transmembrane domains, linked alternately by intra- and extracellular sections. Pendrin participates in active, sodium-dependent transportation of anions, such as: iodides, chlorides, and bicarbonates [8].

The occurrence of pendrin as a monomeric glycoprotein is found in its highest concentrations in the thyroid follicular cells, especially at their colloid adjoining apical surface [13, 39]. Pendrin transports iodine beyond the cell where it binds with thyroglobulin and is stored in colloid and used for the synthesis of thyroid hormones. *SLC26A4* mutations are associated with loss of the protein ability to transport iodides and with abnormal pendrin localisation in cellular cytoplasm [37, 43]. It has been found that the degree of pendrin expression in the thyroid is not closely related with the functional activity of the gland [32] (Fig. 2).

Pendrin transports ions also within the ear. The complex function of the internal ear, including sound wave transformations and linear and angular accelerations into nervous impulses, depends on the presence of rest potential in cochlear and vestibular structures and on strictly determined electrolyte conditions in labyrinthine fluids. The *SLC26A4* gene undergoes expression within the labyrinth mainly in the regions, responsible for endolymph production, i.e., in the *stria vascularis*, the external spiral sulcus and the adjacent part of the spiral ligament, in Hensen's cells, the spiral eminence and in the utricle. Based on immunohistochemical methods, its presence is also identified in Corti's organ – the internal and external hair (auditory) cells, especially in their covering membranes, in supporting cells and the spiral ganglion of the cochlea [51]. In these structures of the internal ear, pendrin participates in HCO_3^- secretion into endolymph. It has been documented that in case of mutated, inactive pendrin, endolymph is acidified in result of decreased HCO_3^- concentration [47]. The increase of endolymph pH suppresses the functionality of KCNJ10 potassium channels and TRP5 and TRP6 calcium channels [47, 26]. The KCNJ10 channels, which transport K^+ to endolymph, play a significant role in inducing and maintaining cochlear rest potential. The KCNJ10 channels are located in intermediary cells, in the *stria vascularis* [46]. The perception of acoustic impulses requires low Ca^{2+} concentrations in endolymph.

The TRP5 and TRP6 epithelial calcium channels occur in the vestibular system and are responsible for Ca^{2+} reabsorption from endolymph [26]. The loss of internal ear functionality, observed in PS and EVAS, results from endolymph acidification, leading to K^+ concentration increase and fall of the intracochlear potential. It also leads to elevated Ca^{2+} concentrations and to loss of the hair cell sensitivity [46]. Using an animal model with murine cell line, deprived of any functional copy of *SLC26A4* gene, degeneration of the *stria vascularis* was found with hyperpigmentation and disorganisation of marginal cells, with secondary infiltration of macrophages [20] (Fig. 3.).

Regarding the kidneys, pendrin occurs in inclusive cells of the cortical collective tubules, where it takes part in the absorption of chlorides and secretion of bicarbonates [38]. The fact that no renal functional disorders are observed in the clinical picture indicates the existence of some pendrin-independent mechanisms of bicarbonate secretion.

Recently, experimental studies have been evaluating the role of pendrin in the renal process of blood pressure control and of arterial blood pH [45].

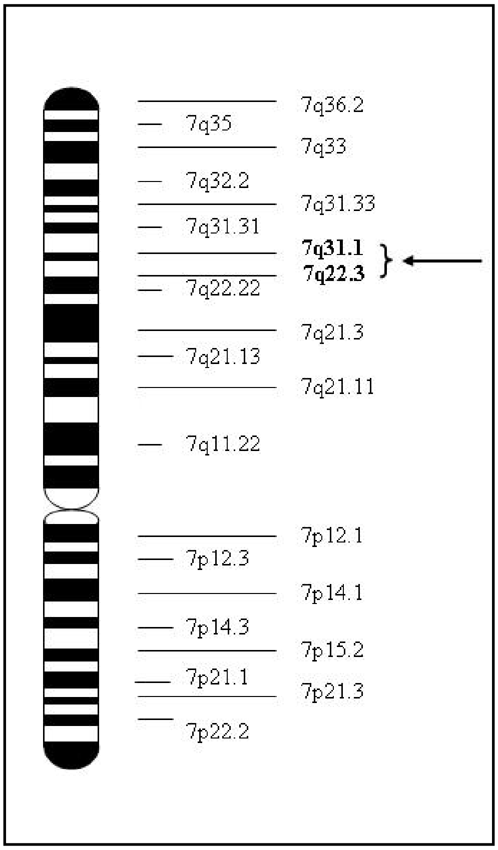

Fig. 1. Location of *SLC26A4* gene in long arm of chromosome 7

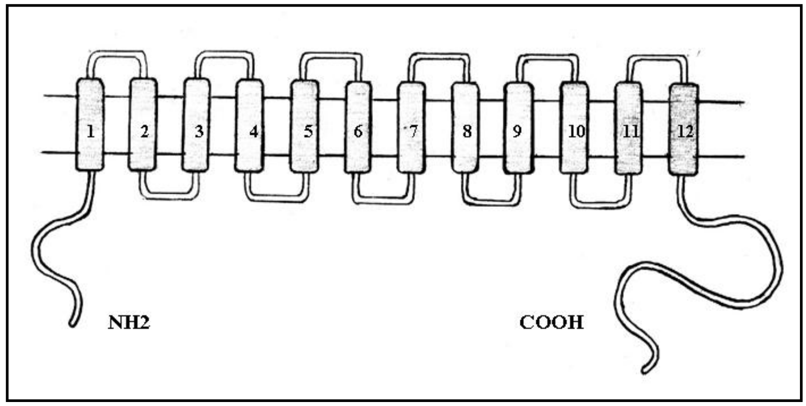

Fig. 2. A model of pendrin molecule

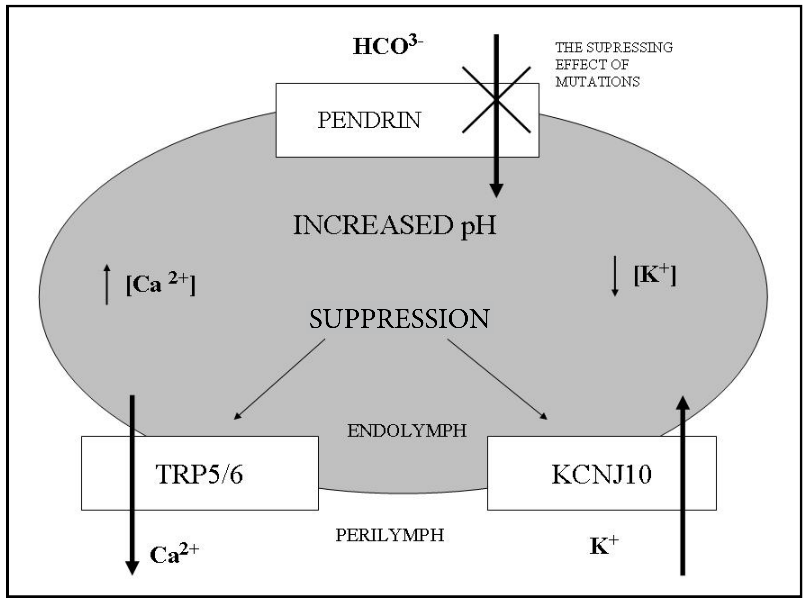

Fig. 3. Disturbances of homeostasis of the internal ear fluids in PS and EVAS

The specificity of pendrin activity in particular organs may result from the existence of its isotypes. It is possible that differences among these isotypes should be looked for beyond the epitope region for the actual antibodies and that is, perhaps, why they have not been identified so far. Another hypothesis, trying to explain it, assumes that pendrin is a component of a multiprotein complex, standing for a tonus-dependent channel for anions. Then, consequently, any differences in the other proteins, included in the structure of this complex, would control the selectivity for particular anions [48].

THE CLINICAL PICTURE

As it has been mentioned before, PS and EVAS are autosomally and recessively inherited diseases. It means that parents, whose child bears the disease, are associated with a 25% risk that another child will also have the same disease. They have also a 50% chance that the next child will be normal, being, however, a carrier of defective gene, and a 25% chance to have another baby which will not be either ill or carrier of the defective gene.

Disorders of hearing and of the balancing system

Hypoacusis in PS and EVAS occurs at the stage prior to speech formation and enhances in the course of speech development, revealing a progressive character in later stages of life. Sometimes, the degree of hypoacusis undergoes certain fluctuations. In general, hypoacusis is manifested as a sensorineural hearing loss, while only rarely with the transmission component [11]. Certain researchers try to explain the interaural difference in hypoacusis by asymmetry in the degree of labyrinth structure disorders [11, 24]. The cases of hypoacusis and genetic deafness are divided into isolated and concomitant with pathological symptoms in disease syndromes. PS is the most frequent genetic syndrome, causing 5–10% of cases of inherited deafness [11, 28, 35]. There are many genetic causes at the base of inherited isolated hypoacusis, which are difficult, if not totally impossible to be identified from the clinical point of view. Hypoacusis, resulting from disorder of *SLC26A4*

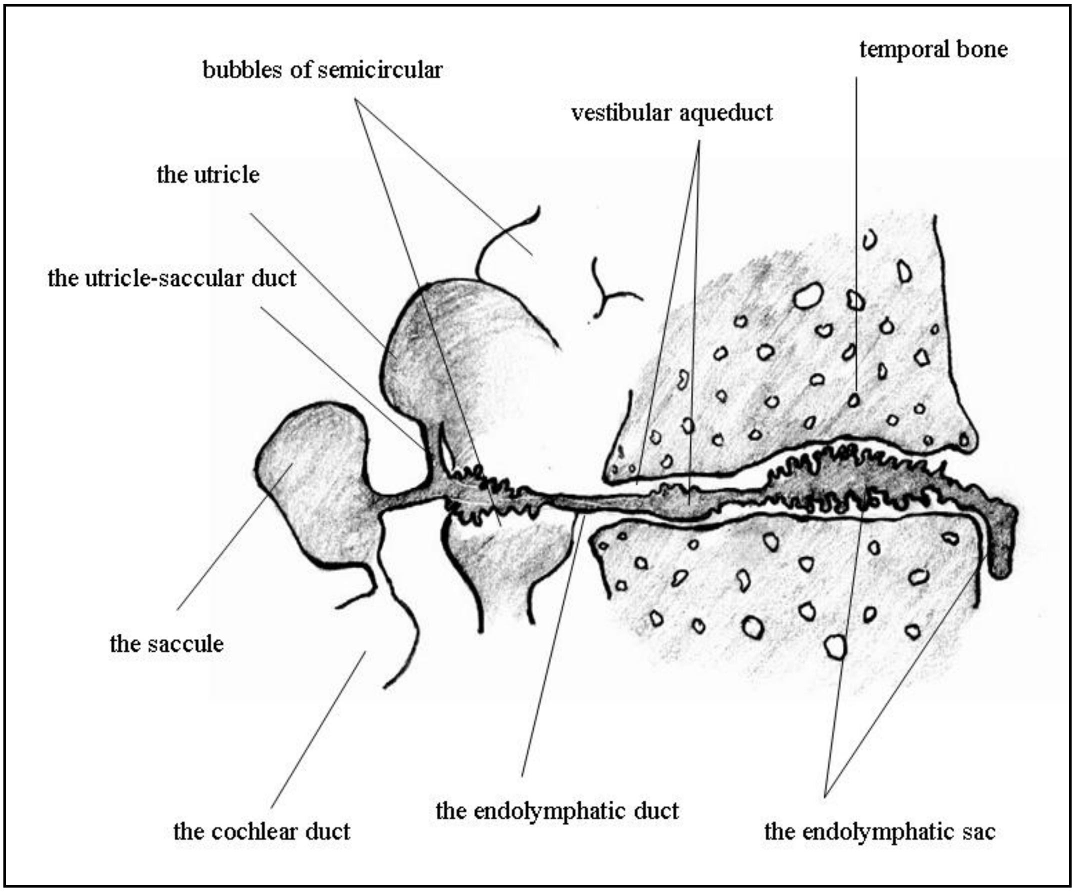

Fig. 4. The endolymphatic duct

gene, is distinguished by the abnormalities of the internal ear structure [6]. Fairly interesting are the results of audiological analysis, combined with radiological evaluation and a 9-year observation of 27 patients with PS and EVAS [5]. Almost the same numbers of persons from each group (about 30%) reported hypoacusis either of steady, progressive character or with stepping increase. Patients with PS had much deeper degree of hypoacusis [5]. No relationship was found between the degree of enlargement of the vestibular aqueduct and the stage of hypoacusis [5]. That observation has also been confirmed by other reports [27]. Dysfunction of the vestibular organ is observed in more than 50% of persons with PS – ranging from light, unilateral channel paresis to bilateral areactivity [35]. It has been found that patients with PS and vertigo more often manifest fluctuating hypoacusis with tinnitus. These subjects, who are homozygotes of *H723R* mutation, experience vertigo episodes much more often than heterozygotes [42].

Endocrine disorders

The defect of iodine organification in the thyroid is, in the clinical practice, identified in a test with potassium perchlorate (PPT). The potassium perchlorate test has been recognised as unreliable because of its low specificity and considerable differences among the criteria for its interpretation. Abnormal PPT results are also found in patients with Hashimoto's disease, Graves' disease and with thyroid peroxidase (TPO) deficit in the gland, following earlier surgical intervention or after ^{131}I radioiodotherapy, also following long-term rich iodine supplementation in food [48]. Iodine organification leads – only in some cases – to slight suppression of thyroid hormone synthesis. For this reason, the screening examinations of newborns are not always effective to identify potential patients [31].

The goitre occurs in 73–80% of persons with PS, however, it is most often observed already in the second decade of life, and no disorders of thyroid hormone concentrations are usually observed [11, 35]. The goitre occurs despite applied therapy with thyroid hormones [22].

Anatomical anomalies

The vestibular aqueduct is a bone canal (diameter = 0.25 mm), localised in the pyramid of the temporal bone. It begins with an internal foramen in the elliptic recess section of the medial labyrinth wall and turns backwards on a 7–9 mm section, towards the posterior pyramid wall, where it is terminated with a slit-like external foramen. In the vestibular aqueduct, there is one of the five (5) main parts of the membranous labyrinth (beside the utricle, the saccule, the cochlear duct and the semicircular canals), namely the endolymphatic duct. This duct, leading out of the saccule, joins the utricle-saccular duct in the vestibular area. At this location, there are folds, separating – to some degree – the cochlear endolymphatic system from the semicircular ducts. Immediately after that juncture, the endolymphatic duct expands twice, forming, so-called – sinuses. Two thirds of the duct is located in the vestibular aqueduct, the terminal part of which extends to contain the third expansion, called the endolymphatic sac. The endolymphatic sac is an expansion, resulting not from inflation and diameter increase but from irregular folding of its inner surface, lined with cuboidal epithelium. The sinuses and the endolymphatic sac participate in the production and resorption of endolymph [23] (Fig. 4).

Enlargement of the vestibular aqueduct is identified when its diameter in the middle of the section between the common limb and the external foramen of the duct amounts to 1.5 mm. Identification of this anomaly is possible by CT or MRI scanning. Enlargement of the vestibular duct, including its content, is the most frequently observed malformation, recognised in the radiological diagnostics of sensorineural hearing loss. This malformation is diagnosed in as many as 12% of children with deafness [1] and in 80–100% with PS [14, 36]. This defect may coexist with incomplete cochlear development, i.e., the lack of apical turns, being referred to in this complex form as Mondini's malformation. Referring to the more and more common use of cochlear implants, CT of the internal ear becomes a routine diagnostic procedure in a growing number of persons with hearing loss, thus resulting in more and more frequent diagnosis of EVAS. Unlike other structural disorders of the internal ear, only EVAS and Mondini's malformation result from *SLC26A4* gene mutations [10]. Other types of labyrinth structure disorders, such as, for example, enlargement of the endolymphatic fossa, hypoplasia of the modiolus or of the horizontal semicircular canal are characteristic for mutations of *GJB2*, encoding conexin-26 protein. These mutations are at the base of more than 50% of cases of autosomally recessive, isolated hypoacusis [2, 33].

CORRELATION OF *SLC26A4* MUTATIONS AND PHENOTYPE

The *SLC26A4* gene is the only, documented so far gene, responsible for PS and EVAS occurrence. At present, the Database of Human Gene Mutations provides 124 recessive mutations of *SLC26A4* gene (Table 1) [19]. These are most often missense (replacement of a single nucleotide by another one, causing stop codon formation – prematurely terminating translations and causing shortened protein chain) and nonsense (replacement of a single nucleotide, causing codon sense modification and, in consequence, incorporation of another, wrong amino acid) mutations. Other types include splicing mutations small insertions and deletions of gene section and rearrangements (inversions). Because of the dispersed localisation of mutations in the gene, all the 21 exons should undergo sequencing to obtain complete genetic diagnostics [34]. Mutations in *SLC26A4* gene are phenotypically heterogenous, which means the occurrence of various clinical symptoms in carriers of the same mutation. Patients in mutations in *SLC26A4* represent at least two types of phenotypes – PS and, more rarely, EVAS. In either disease syndrome, disorders of the internal structure and hearing loss are observed. The factor, which differentiates PS from EVAS, is rather disturbed iodine organification than the presence of thyroid goitre, which is a not entirely penetrating feature in PS. Differential diagnostics of these diseases is rather difficult, especially in childhood. The search for *SLC26A4* mutation pattern, which would provide or confirm substantial clinical diagnosis, has been attempted for 10 years and is further continued (Tab. 1).

Table 1. Type and number of SLC26A4 mutations, as well as of different phenotypes [19] (modified).

Mutation type	The number of identified mutations
missense & nonsense	77
splicing	21
small deletions	15
small insertions	9
rearrangements	2
Phenotype	The number of cases
PS	68
EVAS	17

The detectability of *SLC26A4* gene mutations in PS and EVAS amounts to 90% and 70–78%, respectively [3, 44]. Both in persons with enlarged vestibular aqueduct and in those with Mondini's malformation, *SLC26A4* gene mutations are found in very high percents (70–86%) of studied patients [10, 36, 49]. In other reports, mutations could not be found in 1/3 of the cases of enlarged vestibular aqueduct, while in another one third, only a single mutation of *SLC26A4* allele mutation was identified [15]. The close similarity of clinical pictures and the occurrence of mutations in the same gene prompted some researchers to acknowledge PS and EVAS as two variants of the same disease [44]. So far, no correlation has been found between the type of *SLC26A4* mutation and the degree and type of abnormal formation of the labyrinth structures [25]. Genetic studies of patients with PS and EVAS demonstrate differences in the spectrum of *SLC26A4* gene mutations, depending on the race (white or yellow) of the studied group. The most frequent mutations for the white race subjects (L236P, T416P, IVS8+1G-A) are rare for the Japanese, for whom, H723R exceeds 50% of *SLC26A4* mutations. Therefore, the ethnic background should be taken into account when searching for mutations in this gene [28, 44].

The *SLC26A4* mutations are responsible for 5–10% cases of congenital hearing loss. It has been confirmed in experimental studies that frequent mutations in PS, such as L236P, T416P or E384G cause a complete loss of pendrin dependent transportation of iodides and chlorides, while the mutations, found in EVAS: V480D, V653A, G497S provided maintenance of transportation of these ions, however, at a much lower level. A conclusion has been drawn from the study that this decreased activity of pendrin is satisfactory to eliminate thyroid goitre and disturbances of its functioning from the clinical picture [40].

A significant correlation was found between the phenotype and genotype of SLC26A4, while studying a group of 30 persons with enlarged vestibular aqueduct. In the study, the restrictively defined conditions of interpretation of the test with potassium perchlorate were especially emphasised, looking for reasons for the erroneous differentiation between PS and EVAS just in the clinical difficulty of thyroid phenotype assessment. The presence of single *SLC26A4* mutation concerned 61% of patients with EVAS. All the persons fulfilling the PS criteria had two mutated alleles in *SLC26A4* gene [34]. Also patients with PS turned out in other studies to be either homozygotes or compound (double) heterozygotes [4, 10]. Probably, finding of a single heterozygotic mutation in *SLC26A4* gene in patients with PS results from imperfection of the applied diagnostic methods and should encourage to perform further molecular studies [30]. A report is fairly interesting in which the same *T416P* mutation, occurring in three siblings, coexisted with various degrees of hearing loss of different clinical course and accompanied the development of different disorders of the labyrinth structure [27]. It may be explained by the presence of other mutations or by participation of environmental factors.

The failure in finding *SLC26A4* gene mutations in PS and EVAS probably results from limitations of the molecular techniques, applied for their identification, although it may also be an effect of participation of other genes in their aetiology.

FOXI 1 gene

The latest studies of Yang et al. [50] indicate that a gene of the *FOXI 1* (MIM 60 1093) transcriptive factor may participate in PS and EVAS pathogenesis. The *FOXI 1* gene is localised on the long arm of chromosome 5 (5q34). The studies of Larsson et al. [21] have provided evidence that the *FOXI 1* gene undergoes expression during embryonic development within the ectodermal, otic vesicle, which then develops into the membranous labyrinth. This gene is an early controller of the otic vesicle cell differentiation process into structures of the internal ear [21]. Studies on homozygotic mice with mutated *FOXI 1* gene showed the animals to be deaf, revealing dysfunction of the static system and considerable disorders in the internal ear structure [17]. In the course of later studies, performed on an analogous animal model, a total lack of pendrin, the product of *SLC26A4* gene expression, was found in the epithelial cells of the endolymph duct and sac [18]. It was, in this way, demonstrated that the product of *FOXI 1* gene expression, as a transcriptive factor, controls the efficacy of *SLC26A4* gene transcription process. Yang et al. [50] have performed the first genetic studies, associating *FOXI 1* gene mutations with PS and EVAS in humans. In their research, they have identified a promotor fragment of *SLC26A4* gene, associated with the factor activating its transcription – i.e., with FOXI 1. In nine patients with PS and EVAS, they recognised a new mutation – 103 T-C – concerning just the fragment of *SLC26A4* gene. In other six patients, they demonstrated the presence of *FOXI 1* gene mutations, which prevented the activation of *SLC26A4* gene transcription. The members of one of the families with EVAS, included in the authors' studies, had both genes, i.e., *FOXI 1* and *SLC26A4*, mutated. Following the results of their studies, the authors have developed a hypothesis that both FOXI 1 transcriptive factor gene mutations and mutations of *SLC26A4* gene, subordinate to FOXI 1, are involved in the pathogenesis of PS and EVAS, which has provided the first examples of bigene inheritance being the cause of hypoacusis.

CONCLUDING COMMENTS

A complex clinical evaluation: endocrine and audiological, together with radiological diagnostic imaging, supported by molecular studies of *SLC26A4* gene, are the procedures, necessary for complete and accurate diagnosis of PS and EVAS. Routine molecular diagnostics, available in European and American laboratories, involves a screening examination, searching the three most common mutations. When the mutations are not identified, a panel of 41 known mutations is applied and, eventually, sequencing of all the 21 exons of *SLC26A4* gene is possible in order to find any mutations at all. However, as it has already been mentioned, the routine diagnostics of *SLC26A4* gene allows for an identification of mutations in approximately 50% of patients with PS and EVAS. It is associated with limitations of the sequencing method, which does not identify all the defects of the cutting points, regulatory sequences of the gene or changes in introns (it is assumed, at present, that a sequence of introns influences correct functioning of the gene). It is not, however, excluded that changes in the genome, other than *SLC26A4* mutation, may be at the base of the pathologies. Taking into account the considerable phenotypic similarity of both syndromes and the defect of the same gene, being at the base of the syndromes, some researchers suggest considering both entities as variants (with or without endocrine symptoms) of the same disease. The application of molecular studies in the diagnostics of Pendred's syndrome and the syndrome of enlarged vestibular aqueduct provides significant data on the genetic aetiology of these diseases. It has been demonstrated that a high percent of cases with PS and EVAS (70–90% in research studies, 50% in routine diagnostics) are caused by mutations of both alleles of SLC26A4 gene, either in homo- or in heterozygotic system. The spectrum of observed mutations is similar for either syndrome, however in EVAS, also missense type mutations are observed, which is not met in PS. In both syndromes, the following two missense mutations: *L236P* and *T416P*, together with the mutation, concerning the abnormal splicing process, i.e., IVS8+1G-A, are most frequently found: in a total of 55% of patients with identified mutation of *SLC26A4* gene. The other 45% of changes in the gene include unique mutations, traced in very few families. Because of the phenotype heterogeneity of *SLC26A4* mutations, the result of molecular study does not yet allow for differentiation of the above-mentioned syndromes between each other. Neither can the finding of a single mutated *SLC26A4* allele be in any way decisive for the diagnosis without clinical evaluation towards PS/EVAS. Most probably, additional genetic factors (mutations and polymorphisms of other genes), as well as environmental factors, are responsible for various expressions of the disease phenotype in members of the families with the same defect of *SLC26A4*. The role of molecular studies will grow up, when the gene therapy becomes genotypically specific.

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